TRINITROBENZENESULFONIC ACID COLITIS INDUCES CHANGES IN THE CONTRACTILE RESPONSE OF CIRCULAR SMOOTH MUSCLE IN THE RAT COLON

1996

HOSSEINI

UNIFORMED SERVICES UNIVERSITY OF THE HEALTH SCIENCES
F. EDWARD HÉBERT SCHOOL OF MEDICINE
4301 JONES BRIDGE ROAD
BETHESDA, MARYLAND 20814-4799

GRADUATE EDUCATION
(301) 295-3913
FAX: (301) 295-6772

TEACHING HOSPITALS
WALTER REED ARMY MEDICAL CENTER
NAVAL HOSPITAL, BETHESDA
MALCOLM GROW AIR FORCE MEDICAL CENTER
WILFORD HALL AIR FORCE MEDICAL CENTER

APPROVAL SHEET

Title of Dissertation: "Trinitrobenzenesulfonic acid colitis induces changes in the contractile response of circular smooth muscle in the rat distal colon"

Name of Candidate: Jeanette M. Hosseini
Doctor of Philosophy Degree
27 March 1996

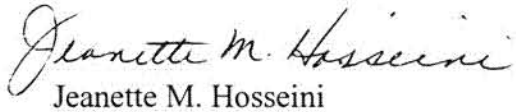
Dissertation and Abstract Approved:

Committee Chairperson	3/27/96 Date
Committee Member	3/27/96 Date
Committee Member	3/27/96 Date
Committee Member	3/27/96 Date
Committee Member	3/27/96 Date

The author hereby certifies that the use of any copyrighted material in the thesis manuscript entitled:

"Trinitrobenzenesulfonic acid colitis induces changes in the contractile response of circular smooth muscle in the rat distal colon"

beyond brief excerpts is with the permission of the copyright owner, and will save and hold harmless the Uniformed Services University of the Health Sciences from any damage which may arise from such copyright violations.



Jeanette M. Hosseini
Department of Physiology
Uniformed Services University of the Health Sciences

ABSTRACT

Title of Dissertation: Trinitrobenzenesulfonic acid colitis induces changes in the contractile response of circular smooth muscle in the rat colon.

Jeanette Marie Hosseini, 1996

Dissertation directed by: Terez Shea-Donohue, Ph.D., Research Associate Professor of Medicine and Physiology.

Diarrhea is a common symptom of colitis. Alterations in colonic muscle contractility, through both neural and smooth muscle mechanisms, contribute to the abnormal motility patterns associated with inflammatory bowel disease. Changing inflammatory milieu may cause stage-dependent alterations of the muscle contractile response. The aim was to study colonic circular smooth muscle response to acetylcholine and the tachykinins during acute and chronic inflammation. Male Sprague-Dawley rats were treated rectally with either saline or trinitrobenzenesulfonic acid in 50% ethanol and studied after four hours (acute) or seven days (chronic). Mucosa-free distal colonic muscle strips were attached to isometric force transducers in the circular axis and placed in organ baths. Concentration response curves were constructed to potassium, tachykinins, acetylcholine, and receptor specific agonists. Muscle from acute animals showed significantly increased responses to potassium (14.7 $\pm$ 2.5 vs. 20.6 $\pm$ 2.7 N/cm^2), substance P (9.7 $\pm$ 0.5 vs. 23.7 $\pm$ 4.5 N/cm^2) and acetylcholine (10.2 $\pm$ 1.3 vs. 17.0 $\pm$ 3.0 N/cm^2), but decreased response to neurokinin A (20.9 $\pm$ 2.3 vs. 13.1 $\pm$1.4 N/cm^2). Acute tissue showed a neural loss in nicotinic-mediated inhibition in response to acetylcholine, as well as changes in tachykinin atropine-sensitivity. The atropine-sensitive changes were

not observed for the selective tachykinin receptor agonists. However, the agonists did demonstrate significantly decreased responses at the level of the muscle for the NK_1 (18.5 $\pm$ 1.5 vs. 11.6 $\pm$ 1.7 N/cm^2), NK_2 (19.8 $\pm$ 1.9 vs. 10.5 $\pm$ 0.7 N/cm^2) and NK_3 (5.7 $\pm$ 1.3 vs. 0.9 $\pm$ 0.4 N/cm^2) receptors. Chronic inflammation decreased non-specific responses, as well as all receptor-mediated events (all $P<0.02$). The progression of the muscle during inflammation from a more contractile state to a less responsive one correlates well with *in vivo* studies showing initial increase followed by a decrease in colonic transit following an inflammatory event. Thus, stage-dependent changes in both nerve and muscle occur during inflammation and may contribute to the diarrhea of colitis.

TRINITROBENZENESULFONIC ACID COLITIS INDUCES CHANGES IN THE CONTRACTILE RESPONSE OF CIRCULAR SMOOTH MUSCLE IN THE RAT COLON

by
Jeanette Marie Hosseini

Dissertation submitted to the Faculty
of the Department of Physiology
Graduate Program of the Uniformed Services University of the
Health Sciences in partial fulfillment of the
Doctor of Philosophy 1996

DEDICATION

"Happy is the man who finds wisdom, and the man who gets understanding, for the gain from it is better than gain from silver and its profit better than gold." (Proverbs 3:13-18)

Scientists, in attempts to understand and make sense of our universe, have often explained away the existence of God. In my personal quest for understanding physiology, I have continually been amazed by the design and function of human body. So much so, that I am confident in the supremacy of our Creator. As much as our civilization has and will advance in understanding, we will never begin to know it all. Isaac Newton was right on target: "*What we know is a drop, what we don't know is an ocean*". Nevertheless, we should continue our pursuit for knowledge of our universe, for it can only bring us closer to God, through admiration of its complexity.

In admiration and appreciation of our creation, this work is dedicated to God and Jesus Christ his son, through which all things are made possible. *"Honor the Lord with your substance and with the first fruits of all your produce"*-(Proverbs 2:9)

May God bless our world with love and peace, which is ours through Christ.

ACKNOWLEDGMENTS

Admittedly, this is the most difficult section to write. Expression of gratitude owed to all who have interacted with me over the last five years requires many more pages than the number contained in this thesis. Everyone in the Physiology Department, including faculty, graduate students, secretarial and technical support staff, have always been there for me when I was in need. Their willingness to help was always en heartening. Staff from other departments, both academic (Pharmacology, Anatomy and Biochemistry) and support (MDL, LRC, LAM and GEO) have been most invaluable, and are all owed my final thanks.

I am grateful for all of the assistance of the people working in the GI section with Terez Shea-Donohue, some of whom have already left, but never the less were instrumental in the beginning stages of my project. Additionally, I have benefited greatly from the expert help and advise of Dr. William Percy, at University of South Dakota. In addition to the human lab members, I feel lucky to have had Poly and Ester (may she rest in peace) to keep me company during late evenings spent in the lab.

I would like to thank the current members of my advisory committee, Dr. Donald Selletti, Dr. Jack McKenzie, Dr. Sheila Muldoon, Dr. James Terris, for their willingness to review and constructively critique my project. I owe an additional thanks to the members of my former committee, Dr. Gregory Mueller, Dr. Patricia Deuster, Dr. Mark Knepper and Dr. Ronald Elin. Both past and present committee members, have not only held a keen interest for my projects, but have been intensely supportive of me, both as a graduate student and as a friend. Dr. Terez Shea-Donohue, my advisor, is the final member of the committee I'd like to thank, because without her, I can truthfully say this wouldn't have happened. Her ability to "take on one more project" has both saved me, and amazed me many times. She has lived up to her name sake of Saint Teresa on many occasions. Her dedication to both research and teaching have been inspirational, and I have gained much from her, both as a advisor and a friend.

Support from family and friends carried me along way during the past five years. My friends, both at school and at NIH have shared both my frustration and elation. I couldn't have had a better support group. My parents, William and Mary Frazier, and my sister, Tina have always been encouraging, supportive, and understanding. They are much of who I am today.

Finally, the greatest expression of gratitude goes to my husband and daughter, for they have had to sacrifice the most of anyone around me. To my daughter, Shahla, I am grateful for her understanding when I couldn't attend a certain function, or live up to her expectations of being a mom. Her support, encouragement and help have given me strength during difficult times. I would like to thank my husband, Mike, who was supportive in my decision to return to school, and has had to take on a greater amount of work as a result. He's carried the ball at home more than I'm willing to admit to him on paper. Often times he couldn't understand my frustrations, but he was there for me anyway. I am grateful for their loving patience, understanding, and support. No one could have been more giving of themselves at a time more demanding.

I regret that I am unable to mention by name, all those who have contributed to my thesis, and I apologize if I have inadvertently overlooked someone in the process. Please know that I am deeply appreciative and will never be able to completely express my gratitude in its entirety.

TABLE OF CONTENTS

I. INTRODUCTION 1

A. Clinical Relevance 1
B. Inflammatory Model 2
C. Enteric Nervous System (ENS) Regulation of Motility 3
D. Smooth Muscle Properties 7
E. Acetylcholine (ACH) Background 9
F. Regulation of ACH Activity 10
G. Tachykinin (TK) Background 10
H. Regulation of TK Activity 11
I. Evidence for TK/Cholinergic changes and the Immunological Role of TKs during Inflammation 11

II. RATIONALE & HYPOTHESIS 17

III. MATERIALS, METHODS AND DESIGN 18

A. Inflammatory Animal Model 18
B. Inflammation/Histology/Muscle Characteristics 18
C. Design 19
D. Muscle Contractility Measurements 22
E. Reagents 22
F. Calculation of Results 23
G. Statistics 24

IV. RESULTS 25

A. Histology, Inflammation and Muscle Parameters 25
    1. Histology and Inflammation 25
    2. Muscle Parameters 25
B. Muscle Contraction Results 34
    1. Investigations of Saline, Acute and Chronic 34
        a. Stage Dependent Changes 34
        b. Cholinergic Influence of TK Response 38
        c. Changes in Cholinergic Mechanisms 48

2. Investigations of Acute Response Changes 52
a. Location of Cholinergic Changes of Endogenous TKs 52
b. Receptor Specific TK Agonists 59
c. Changes in Relative Responses 64
C. Summary of Contraction Results 70

V. DISCUSSION 71
A. Muscle Changes 71
1. Acute Changes 74
2. Chronic Changes 77
B. Neural Changes 78
1. Acute Changes 78
2. Chronic Changes 83
C. Mechanisms for the Opposing Changes of the TKs 83

VI. SUMMARY AND CONCLUSION 90

VII. BIBLIOGRAPHY 91

LIST OF FIGURES

Figure 1	Enteric Nervous System	6
Figure 2	Photomicrographs of HE sections of Saline, Acute and Chronic Treated Distal Colon	28
Figure 3	Photomicrographs of Geimsa sections of Saline, Acute, and Chronic Treated Distal Colon	30
Figure 4	Stage-Dependent Changes in Contractile Response	36
Figure 5	Representative Grass Recordings from Tissue Challenged with 60 mM KCl	41
Figure 6	Saline and Acute Atropine Dependent TK Responses	43
Figure 7	Chronic Atropine Dependent TK Responses	45
Figure 8	Stage-Dependent Changes of TK Responses in Atropine	47
Figure 9	Stage-Dependent Changes of ACH Responses in Atropine and Hexamethonium	50
Figure 10	TTX-Sensitive Neural Regulation of SP Response	55
Figure 11	TTX-Sensitive Neural Regulation of NKA Response	58
Figure 12	TTX-Sensitive Neural Regulation of NK1 Response	63
Figure 13	TTX-Sensitive Neural Regulation of NK2 Response	66

Figure 14 Relative Comparisons of Receptor Selective Agonists and TK Contractile Response 69

Figure 15 Mechanisms Contributing to Muscle Contractility 73

Figure 16 Mechanisms of ENS Neural Component 81

Figure 17 Proposed Hypothetical Model of NKA Atropine Sensitive Neural Influence 86

Figure 18 Proposed Hypothetial Model of SP Atropine-Sensitive Neural Influence 89

LIST OF TABLES

Table I	Inflamed Distal Colonic Contractility	14
Table II	Inflammatory Evaluation Criteria	20
Table III	Muscle Contractility Design	21
Table IV	Inflammatory Scores and Histology Measurements	26
Table V	Physical Characteristics of Muscle Tissue	32
Table VI	Inflammatory Colitis Rat Model	33
Table VII	Maximum Tensions and EC_{50} Values for Saline, Acute and Chronic Groups	37
Table VIII	Maxiumum Tensions and EC_{50} Values for Saline, Acute and Chronic Groups	56

LIST OF ABBREVIATIONS

IBD	Inflammatory bowel disease
TNBS	2,4,6-trinitrobenzenesulfonic acid
ENS	Enteric nervous system
ANS	Autonomic nervous system
CNS	Central nervous system
EPI	Epinephrine
NE	Norepinephrine
SNS	Sympathetic nervous system
PSNS	Parasympathetic nervous system
ACH	Acetylcholine
NANC	Non-cholinergic, non-adrenergic
VIP	Vasoactive inhibitory peptide
NO	Nitric oxide
NKA	Neurokinin A
SP	Substance P
NKB	Neurokinin B
SR	Sarcoplasmic reticulum
BER	Basic electrical rhythm - or slow wave
N	Nicotinic receptor
M_1,M_2,M_3	Muscarinic receptors
HEX	Hexamethonium
TTX	Tetrodotoxin
β-Ala	[β-ala^8]-NKA$^{(4-10)}$-agonist at NK_2 receptor
Sar P	Sar substance P, agonist at NK_1 receptor
Senk	Senktide, agonist at NK_3 receptor
IP_3	Inositol 1,4,5 triphosphate
DAG	Diacylglycerol
cAMP	Cyclic 3',5'- adenosine monophosphate
cGMP	Cyclic guanine monophosphate
CAT	Acetyltransferase

TK	Tachykinin
NK_1	Tackykinin receptor, affinity highest for SP
NK_2	Tackykinin receptor, affinity highest for NKA
NK_3	Tackykinin receptor, affinity highest for NKB
CGRP	Calcitonin gene related peptide
EFS	Electrical field stimulation
EC_{50}	Concentration for 50% of maximal response
T_{max}	Maximal tension

INTRODUCTION

Clinical Relevance

The incidence of inflammatory bowel disease (IBD) such as Crohn's and ulcerative colitis, is between 5-10 per 100,000 in the United States[40]. Ulcerative colitis initially presents with inflammation of the distal colon and progresses in a retrograde fashion to include areas of the colon more proximal[31] It can present as acute, chronic or may reoccur as an acute relapse, and is unpredictable in its course[4]. One well accepted possible etiology of IBD is that the mucosal barrier is somehow compromised permitting entry of a luminal antigen to the lamina propria. It has been suggested that there is an inappropriate response by the mucosal immune system, initiating an uncontrolled cascade of inflammatory events[56, 59]. Acute disease occurs as the body mounts its first response to the invading antigen and is typified by aggregation of platelets, tissue fluid accumulation, vascular permeability changes, epithelial lifting, and a cellular infiltrate primarily characterized by neutrophils and few monocytes, and little or no change in the muscularis externa[44]. The disease progresses into a more chronic immune response with involvement of lymphocytes and the production of specific growth factors and/or cytokines. Characteristic histology of the chronic phase includes hyperplasia and hypertrophy of the muscularis externa, mild vasocongestion, epithelial cell sloughing, and a primarily lymphocytic infiltrate [3, 44, 54]. The two stages can be differentiated histologically using a graded scaling method[54]. While the two stages can be separated easily morphologically in controlled animal models, in practice, the clinician most often encounters an "acute" inflammation in IBD patients subject to relapse of disease. Additionally, it is the acute stage that most frequently causes abdominal discomfort and diarrhea. Therefore, reoccurrence of the acute phase of inflammation (relapse) in the distal colon is the most clinically relevant scenario.

Diarrhea and intestinal cramps are definitive symptoms of IBD and their occurrence is associated with changes in normal motility patterns. Normal motility patterns in the colon consist of short-duration, long-duration, and giant migrating contractions[53]. The spatial arrangement and coordination of these movements allow both a slow net distal propulsion that provides uniform mixing and mucosal exposure to a semi-solid to solid bolus, and a rapid propulsion in the caudad direction for mass movements and defecation, respectively[52, 53]. Both short and long duration contractions (amplitude and duration) are either reduced or absent in colitis, with the severity of diarrhea correlating positively with the degree of reduction in these patterns[53]. Additionally, in both animal models and humans, there is an increase in giant migrating contractions during colitis[53]. Decreased long and short duration contractions reduce mixing time and exposure of the colonic contents to the mucosa and thus decrease fluid absorption. In contrast, increases in giant migrating contractions initiate a mass movement of colonic contents and the defecation reflex. Changes in either or both motility patterns contribute to the diarrhea and cramping experienced with colitis.

Inflammatory Model

Numerous animal models have been developed to study IBD. A well accepted model to study both the acute and chronic changes associated with IBD was originally developed by Morris et. al. in rats using ethanol and TNBS (2,4,6-trinitrobenzenesulfonic acid)[42]. TNBS, a haptan, is administered in ethanol to disrupt the mucosal barrier and combines with gut proteins to become antigenic[42]. Additionally, metabolism of TNBS creates reactive oxidants, which facilitate the inflammatory process[64]. When compared to acetic acid induced inflammation, TNBS gives a more consistent and long-lasting inflammatory pattern typical of the acute stages of IBD. By two weeks, the affected area subsequently develops characteristics of chronic inflammation [65]. Acute changes seen within four hours

in the TNBS model include increases in smooth muscle spike activity, mucosal permeability, edema, and defense cell (neutrophils) infiltration, mucosal hemorrhage, and eicosanoid production[57, 65]. Moreover, during the acute stage, TNBS models show increased motility mediated in part by the lipoxygenase product of arachadonic acid, LTD_4[46]. Chronic changes seen in rat colon with the TNBS model include thickening of the muscularis externa, lymphocytic infiltration, and decreased contractility[42]. Therefore the TNBS/ethanol method provides an experimental model for the study of both acute and chronic stages of colitis that closely correlates with stages of inflammation seen clinically.

Enteric Nervous System (ENS) regulation of Motility

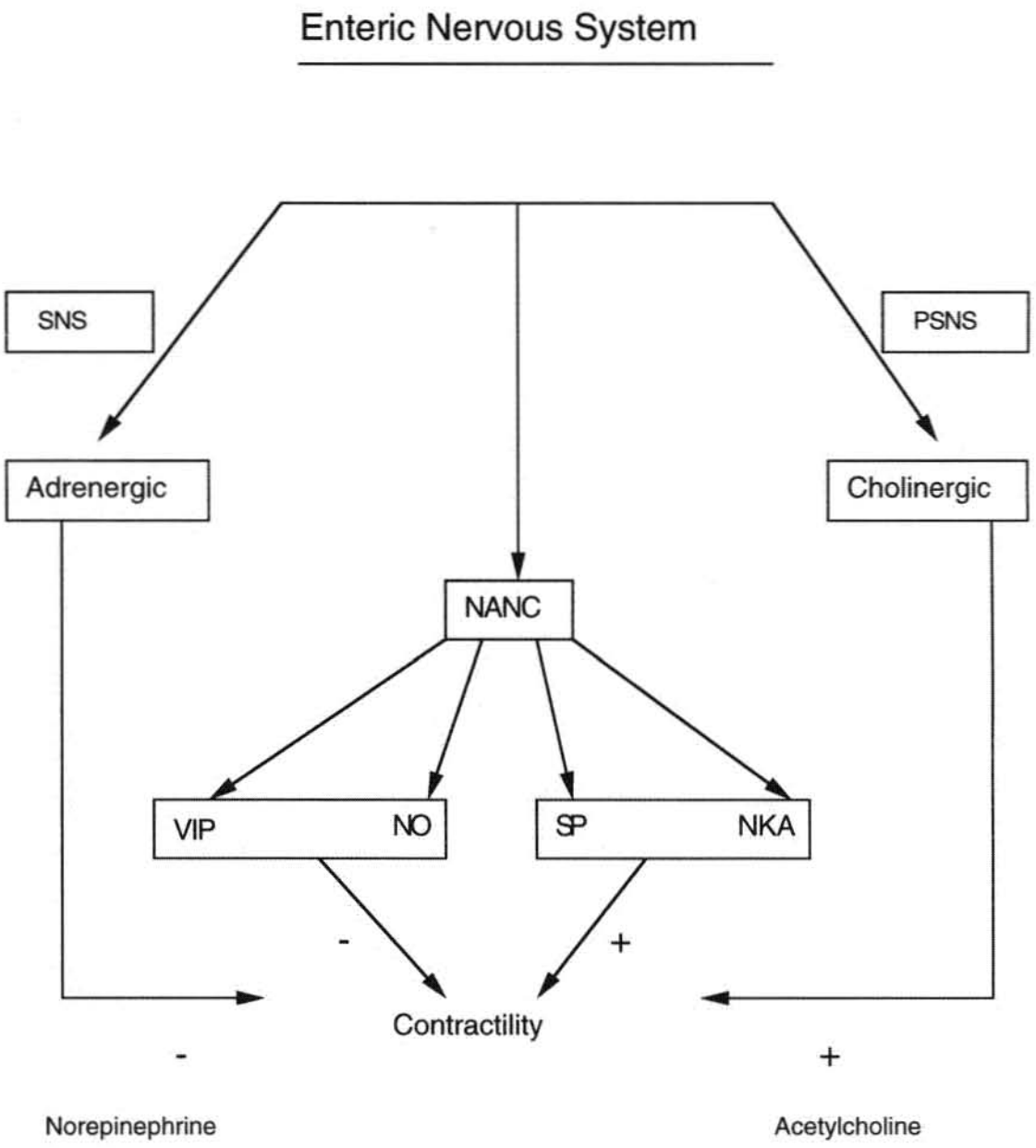
It is known that abnormal motility results from changes in smooth muscle contractility during inflammation. Contractility is determined by: 1) the "net" neural input to the muscle and 2) inherent smooth muscle properties. The hierarchy of neural influence is such that intrinsic nerves in the wall of the gut that comprise the enteric nervous system (ENS) form the first level of regulation of motility, over both the extrinsic autonomic (ANS) and central nervous systems (CNS). The ENS consists of two plexuses, one beneath the epithelial layer, known as the submucosal complex, and a second that lies between the circular and longitudinal muscle layers of the muscularis externa called the myenteric plexus. The muscularis externa is primarily regulated by the myenteric plexus. Extrinsic input to the ENS is via the ANS by either the sympathetic nervous system (SNS) releasing norepinephrine (NE) from post ganglionic neurons or by parasympathetic nerves (PSNS) releasing acetylcholine (ACH) onto preganglionic neurons within the plexus. Both the CNS and ANS have a relatively small role in the control of motility under most conditions. Additionally, local sensory information is received in the ENS from both mucosal and muscle sensory receptors. Within the ENS, there are motor and sensory

neurons, as well as interneurons that integrate and coordinate neuronal signaling.

The ENS has cholinergic, adrengeric, and non-adrenergic non-cholinergic, (NANC) neurons (Figure 1) that may undergo changes in number, connection, or function in IBD. While adrenergic neurotransmitters are inhibitory to smooth muscle, the more important changes in inhibitory neurotransmission during inflammation appear to involve the NANC containing neurons within the ENS. Although there are a number of putative NANC neurotransmitters, the major physiological transmitters are the tachykinins (substance P and neurokinin A), vasoactive inhibitory peptide (VIP) and nitric oxide (NO). Contractility of smooth muscle is the net result of all motor neuron transmitters released, both inhibitory and excitatory. Neurokinin A (NKA) and substance P (SP) increase smooth muscle contractility, while NO and VIP relax smooth muscle (Figure 1). Additionally, adrenergic, cholinergic and NANC neurotransmitters can influence interneurons or motor neurons in either an inhibitory or excitatory fashion through inter neuronal connections. Output from the ENS to the local effector organ, the muscle, controls the minute to minute coordination of contractions in time and space.

Figure 1 Enteric nervous system regulation of contractility in the colon

Figure 1


Enteric Nervous System
SNS
PSNS
Adrenergic
Cholinergic
NANC
VIP
NO
SP
NKA
-
+
Contractility
-
+
Norepinephrine
Acetylcholine

Smooth Muscle Properties

Smooth muscle contraction, which underlies all motility patterns, is in part defined by properties inherent in the smooth muscle. Smooth muscle in vascular tissue, as well as the gastrointestinal tract, exhibits characteristics that are both similar and different from those of skeletal muscle. Both muscle types rely on intracellular calcium (Ca^{+2}) for interaction of actin and myosin to produce a contraction. In both types of muscle, this interaction between actin and myosin proteins can be optimized by the degree the muscle is stretched (length) prior to the contraction in both muscle types. The degree of stretch that enables maximum exposure of actin-myosin contact points (or "crossbridge formation") provides the greatest contractile force when challenged with a depolarizing stimulus, and is defined as the optimal length (L_O). The tension developed within the tissue prior to the contractile event is termed passive tension, the tension developed in response to the stimulation is termed active tension, and the sum of the two is the total tension.

Smooth muscle differs from skeletal in the arrangement and relative amounts of the contractile proteins actin and myosin. While skeletal muscle actin and myosin are associated in a orderly parallel fashion, with actin molecules anchored to the cell wall at the z lines, the association of actin and myosin in smooth muscle is more randomly oriented, and actin filaments are attached to the cell wall at z-line protein containing junctions known as dense bodies.

Smooth muscle differs from skeletal muscle in the source of Ca^{+2}. Smooth muscle does not have the extensive sarcoplasmic reticulum (SR) stores of intracellular Ca^{+2} and therefore, relies much more on extracellular Ca^{+2} influx for Ca^{+2} induced contraction. Ca^{+2} influx occurs following the opening of Ca^{+2} channels either on the SR or plasma membrane and can be either receptor mediated (receptor activated channels) or can occur as the depolarizing stimulus opens Ca^{+2} channels (voltage sensitive channels), such as when large amounts of extracellular potassium are applied to the cell membrane. The relative proportion of extracellular to intracellular fluxes

appears to vary according to the anatomical location of the smooth muscle within the gut. Longitudinal smooth muscle relies more on extracellular Ca^{+2} influx than circular muscle and circular muscle more on intracellular release of Ca^{+2} than longitudinal[24].

Smooth muscle exhibits differences from skeletal muscle in both the neural initiation of contraction, as well as the contractile process. The process of contraction in skeletal muscle relies on potentials developed in response to neurotransmitter release at specialized structures known as endplates, initiating a depolarization of the t-tubule system which results in an "all or none" contraction. This contraction results from interaction between actin and myosin which is dependent on the presence of troponin and tropomyosin. In contrast, smooth muscle, influenced by neurotransmitter release from varicosities, is a calmodulin-dependent process and the degree of myosin light chain kinase phosphorylation determines the degree of contraction elicited, promoting a "graded" response. As such, because the smooth muscle cells are connected by gap junctions, the smooth muscle layer exhibits a graded contraction or "tone" while under the influence of the ENS.

The circular smooth muscle layer contains specialized cells that exhibit a periodic fluctuation of their membrane potential and they drive other muscle cells connected to them by gap junctions to experience the same fluctuations. These cells, known as the Interstitial cells of Cajal, effectively generate what is known as the basic electrical rhythm (BER) of the gut. The muscle may reach threshold at the peak of one of these depolarizing cycles and contract depending on the environment at the time.

In this respect, gastrointestinal muscle and vascular muscle differ because vascular muscle doesn't exhibit BER. Additionally, while vascular smooth muscle requires stimulatory input from the SNS to maintain contractile tone, the gastrointestinal smooth muscle is inherently contractile and depends upon a continuous inhibitory input from the ENS. The importance of inhibitory input is shown by the powerful almost "tetanic" contraction of the gut in the absence of the ENS.

Therefore, not only neural input, but the sum of both neural and inherent smooth muscle characteristics defines the contractile state. Thus, in the course of IBD, it is possible that immunological priming of muscle or neurons impacts on muscle contractility to change: 1) receptor density or receptor affinity, 2) post-receptor signaling mechanisms, 3) release or degradation of excitatory or inhibitory transmitter, 4) muscle or neuronal permeability (excitability) 5) neuronal associations 6) or contractile protein amount or efficiency. Any or all of these can undergo changes due to the inflammatory milieu and work together to produce the motility patterns characteristic of IBD.

Acetylcholine (ACH) Background

ACH is one of the major neurotransmitters of the ANS. It is involved in stimulation of all autonomic ganglia, and it is released from pre- and postganglionic neurons of the PSNS. ACH acts on both nerve and smooth muscle in the GI tract through muscarinic (M) and nicotinic receptors (N). N receptor activation causes ligand-gated cation channels to open and depolarize the cell. Within the wall of the colon, N receptors are present only on the ganglionic neurons, while M receptors are present on ganglionic neurons, as well as smooth muscle. Action at N receptors can be effectively blocked by hexamethonium (HEX), a non-depolarizing ganglionic blocker that acts to physically block the N regulated cation channel.

The three major M receptor subtypes and their locations within the colon are: M_1, primarily on the post synaptic nerve terminals; M_2, on presynaptic terminals of nerves; and M_3, present on the glandular and smooth muscle tissue [47]. All three types of M receptors are effectively blocked by atropine, a competitive antagonist of ACH. M_1 and M_3 receptor activation cause an increase in inositol 1,4,5 triphosphate (IP_3) and diacylglycerol (DAG) second messengers that either decrease potassium conductance (M_1) or increase intracellular Ca^{+2} (M_3), stimulating a response[47]. M_2 receptors, located presynaptically, mediate a decrease in cAMP, increased potassium conductance and hyperpolarization of the

neuron responsible for release of the activating transmitter (ACH)[47]. Therefore, M_2 receptors are involved in negative feedback mechanisms regulating ACH release.

Regulation of ACH activity

In addition to the auto-inhibitory feedback mediated by M_2 receptors, ACH release is inhibited by NE at the presynaptic terminal, also decreasing further release[47]. ACH activity is regulated by limitations in synthesis and degradation. Choline is acylated by acetyltransferase (CAT) in the production of ACH. The rate limiting step in the production of ACH is choline transport into the cell (which in turn is controlled by the release of ACH). Cholinesterase, located on the presynaptic membrane, hydrolyzes ACH, effectively deactivating the molecule. Finally, yet another way ACH activity may be regulated is through the co-release of other transmitters or modulatory peptides, which may be either synergistic or inhibitory in nature[47].

Tachykinin (TK) Background

TKs are a family of bioactive peptides that share a common carboxyl-terminal sequence and thus exhibit similar pharmacological properties [43]. The three mammalian TKs and their respective preferred receptors are; SP: NK-1, NKA: NK-2, and Neurokinin B(NKB): NK-3[23]. Only the endogenous TKs, SP and NKA, are relevant to IBD, since NKB is absent in the peripheral nervous tissue[23]. Both SP and NKA exhibit cross reactivity for the three receptors that are located on both enteric neurons and smooth muscle cells within the gut [1, 23]. The relative distribution of the types between muscle and nerve varies however, between species and by location along the gastrointestinal tract[26]. In general, NK-1 receptors are located on both nerve and muscle, while NK-2 is primarily (but not exclusively) on muscle, and NK-3 is primarily (but not exclusively) on nerves[1, 36]. Furthermore, SP is more effective at releasing ACH from enteric neurons, while NKA has a more direct action on smooth muscle [23].

SP and NKA are present in the nerve endings of the ENS and act on both muscle and nerve to cause depolarizing events. Activation of any of the three TK receptors on smooth muscle, through associated G proteins, increases IP_3 and DAG second messenger pathways causing increased release of intracellular Ca^{+2} to activate contractile mechanisms [26, 34]. TKs effectively depolarize myenteric neurons in a dose dependent fashion primarily by causing increased membrane resistance to potassium current[15]. TKs can therefore excite smooth muscle, both directly, and indirectly through neuromodulation of other enteric neurons [27].

Regulation of TK activity

Clearance of the TKs from the neural synapse occurs through action of neutral endopeptidases. As a result, changes in these endopeptidases may modulate effects of the TKs, similar to the degradative enzymes of the cholinergic and adrenergic systems [9]. Neurotransmitter activity may be shut down by desensitization, a process known as tachyphylaxis. This process regulates the SP, but not the NKA response.

The TKs both modulate, and are modulated by, the release of ACH [10, 23, 28, 41, 55]. Cholinergic TK influence has been reported to be both inhibitory and synergistic [32, 55]. It is well documented that ACH co-exists with SP in nerve terminals and ACH release decreases SP release by presynaptic inhibitory mechanisms [10, 41, 55]. Therefore, changes in either transmitter pathway could impact on the other during the inflammatory process.

Evidence for TK/Cholinergic changes and the immunological role of TKs during inflammation

Current *in vivo* evidence suggests that TK neurotransmitters, the major NANC contractile agents in the ENS, may contribute to motility changes associated with IBD. Immunocytochemical localization of SP in colonic tissue from Crohn's and ulcerative colitis

patients indicates there is an elevated production of TKs in both diseases [39]. SP increases are seen along the basolateral areas of the epithelial cells and in the circular smooth muscle layer. In Crohn's disease, SP is elevated in the longitudinal muscle, an area normally containing only a sparse number of SP-containing neurons [39]. Additionally, NK_1 receptors are significantly increased in colon from IBD patients[37].

TKs are intimately associated with the immunological system. SP, along with calcitonin gene related peptide (CGRP), is present in capsaicin-sensitive afferent C fibers [29]. Capsaicin, the pungent ingredient of red pepper, acts on unmyelinated C fibers. It has been used as a tool to evaluate the contribution of primary afferent neurons. Capsaicin acts at a "vanilloid" receptor which is expressed by a subset of afferent neurons designated "capsaicin sensitive primary afferents".

Acute administration of capsaicin selectively activates sensory afferents to release primarily SP, VIP and CGRP. This effect is transient, not reproducible, and protective. SP may mediate signals that increase protection (by way of increased blood flow or immuno-modulation) to the damaged mucosa [29]. Acute administration of capsaicin with colitis induction in two animal models (rabbit and rat) showed a protective effect [22, 49].

Chronic administration of capsaisin depletes SP and CGRP from afferent nerve terminals, desensitizes sensory afferents and thus enhances the effects of noxious stimuli. Chronic pre-administration of capsaicin exacerbated experimental colitis in rats, suggesting that sensory afferents are protective during induction of colitis [11].

TKs act as vasodilators, agonists of intestinal smooth muscle contractility and mucosal secretory agents and are known to impact on a number of inflammatory responses such as mast cell and macrophage recruitment and stimulation [27, 43]. Therefore, changes in the TKs during inflammation may not only serve to immuno-modulate, but they may also cause alterations in neuronal or muscle tissue, contributing to motility disturbances associated with IBD.

Cholinergic neurotransmitters are also thought to contribute to motility changes associated with inflammation. Inflammation appears to cause complex alterations in ACH metabolism at multiple levels. Intestinal inflammation causes a hyperresponsiveness of smooth muscle to carbachol, possibly by a decreased release of ACH from inflamed tissues. Studies of inflamed jejunum have documented there is decreased release of ACH from nerve terminals[7]. Recently, the same group reported inflammatory increases in the activity of acetyltransferase (CAT), the enzyme responsible for the production of ACH[38]. The same study showed a depressed activity of the degradative enzyme acetylcholinesterase in response to inflammation[38]. Both the increased activity of the production enzyme and the decreased activity of the degradative enzyme are maneuvers that would tend to increase the level of ACH at the synaptic terminal. Both the hyperresponsiveness of the muscle to carbachol (possible up-regulation of receptors due to decreased ACH release) and changes in degradative and synthetic pathways may serve as regulatory attempts to increase ACH activity at the muscle. Many investigators suggest that the cholinergic influence of SP may be either inhibitory or synergistic [32, 55]. Therefore, there appear to be alterations in ACH that could influence motility during inflammation either directly or through modulation of SP response.

Changes in muscle contractility of distal colon have been assessed primarily *in vitro* in chronic models of inflammation (>5 days post inflammation) (Table I) [25, 44, 45, 58] The results vary according to the model and species studied. Muscularis mucosae of rabbits inflamed with TNBS exhibited no contractile changes in response to SP, VIP and histamine, but had depressed responses to arachidonic acid metabolites, ACH and KCl[44, 45]. Muscularis externa appears to be unaffected by inflammation in this model[44]. Circular muscle from a different model of inflamed rabbit (formalin/immune complex) showed decreased responsiveness to electrical field stimulation (EFS), bethanechol, and KCl[58]. Inflamed rat longitudinal muscle using TNBS, acetic acid, mitomycin C and *T. spiralis* larvae as inflammatory agents demonstrated depressed

Table I

INFLAMED DISTAL COLONIC CONTRACTILITY

Ref.	Agonists	Model	Muscle	Contractility
(58)	Bethanechol	Rabbit	Circular	↓
	K	Formalin/immune		
		Chronic(5 days)		
(44)	Acetylcholine	Rabbit	Circular	↔
	PGE_2, $PGF2\alpha$	TNBS/50% EtOH	Longitudinal	↔
	LTB_4, LTD_4	Chronic(5 days)	Muscularis mucosae	↓
	K (60mM)			
(45)	VIP	Rabbit	Muscularis mucosae	↔
	SP	TNBS/50% EtOH	"	↔
		Chronic(5 days)		
(25)	Carbachol	Rat	Longitudinal	↓
	SP	TNBS/50% EtOH		
	K	Acetic acid		
		Mitomycin C		
		T. spiralis larvae		
		Chronic(6 days)		

responsiveness to carbachol, SP and KCl in all models[25]. Despite the mixed results from these studies, there appears to be a non-specific reduction in contractility of the colonic musculature in chronic inflammation.

In contrast, an *in vivo* study by Pons et. al., measured colonic transit in a TNBS model of colitis and found an initial increase in transit within the first 8 hours of inflammation, followed by an overall decreased transit during the following 24-48 hours post inflammation[46]. It is of interest to note that TKs are decreased in a time dependent fashion during the acute phase of colitis in rabbits and become significantly reduced after eight hours[12]. These data suggest that the initial stages of inflammation are characterized by TK increase, causing increased contractility and transit. Increased transit may be the result of TK initiated giant migrating contractions typical of colitis. This may be followed by a reduction in TK release, decreased contractility and transit.

Recently, a study in a rabbit model of acute ileitis, showed an enhanced non-cholinergic excitation of ileal circular smooth muscle in response to EFS[19]. Desensitization to SP ameliorated this excitation, indicating that there might be an increase in the release of, or sensitivity to TKs during the acute inflammatory process. Additional studies of this same ileitis model showed changes in NKA muscle contractility as well (submitted for publication). The results support a role for TK-mediated increase in contractility during the initial stages of inflammation.

There is at present little information on TK induced contractility during the acute stages of inflammation in the colon. Additionally, information on cholinergic changes during inflammation is derived primarily from nematode infection-induced inflammation in the intestine, which may or may not be relevant to IBD. Mounting evidence from studies in the small intestine supports the hypothesis that contractile responses to TK transmitters are altered during the acute phase of inflammation (possibly in concert with cholinergic changes) and may contribute to motility disturbances seen in IBD. It is apparent from the literature that there are cholinergic and TK mediated changes during inflammation of the intestine. It is unclear

whether similar alterations of these transmitters exist in the colon, or whether they may differ between acute and chronic stages of inflammation.

RATIONALE:

The present study was designed to investigate: 1) changes in the response of colonic circular smooth muscle to TKs and ACH during both acute and chronic inflammation 2) to discriminate between changes in non-specific contractile properties of the muscle and receptor-mediated changes during the course of the inflammation.

HYPOTHESIS I

There are specific alterations in the circular smooth muscle response to TKs in acute versus chronic inflammation of the distal rat colon.

HYPOTHESIS II

There are alterations in the neural control of smooth muscle response to TKs, as well as in TK receptor function, in acute inflammation of the distal rat colon.

MATERIALS, METHODS, AND DESIGN

Inflammatory Animal model

Male Sprague Dawley rats (purchased from Taconic Farm and weighing between 150-350 g) were anesthetized with Rompun (12 mg/Kg) (Miles Inc., Shawnee Mission, KS) and Ketamine (60 mg/Kg) (Fort Dodge Laboratories Inc., Fort Dodge, IA), for the rectal administration of 1 mL of either TNBS solution (25 mg/mL 50% ethanol) or saline. They were re-anesthetized for surgical removal of the entire distal colon (up to the transverse colon) at the end of the either 4 hours (acute) or 7 days (chronic). Tissue was taken for *in vitro* muscle contracture measurements and histological evaluation. Animals were then euthanized with an overdose of pentabarbital administered intraperitoneally.

Inflammation/histology/muscle characteristics

Full thickness sections of distal colon from each animal were pinned mucosa side up and fixed in 4% formaldehyde and 1% gluteraldehyde in 176 mOsmol buffer. Representative tissues from each group (n=5-7) were embedded in paraffin, sectioned (6 μm in thickness) and stained with either hematoxylin and eosin (H&E) or Giemsa for light microscopic examination. Giemsa-stained slides were used to differentiate leukocyte infiltrate.

In H&E stained sections, an ocular micrometer was used to measure the thickness of the muscularis externa, circular muscularis externa, muscularis mucosae and submucosa using a 20 X objective (Olympus, Olympus Optical Co., LTD, Tokyo, Japan). Three to five measurements of each area were taken from each animal and the values averaged. The ratio of circular muscularis externa to total muscularis externa was used in the calculation of smooth muscle force expressed per cross-sectional surface area. Changes in the

muscularis externa measurement were used as an index of muscle thickening.

Inflammatory scores, based on criteria listed in Table II, were assessed in a blinded fashion to evaluate mucosa, lamina propria, submucosa and muscle structures. Scores for epithelial integrity were based on a single rating of the mucosa using Table II criteria, while scores for vasocongestion were an average of ratings for lamina propria, submucosa and muscle layers.

Using the method described by Percy et. al., the length of maximal active tension development (L_O) was determined for all muscle strips, using ACH (10^{-4} M)[44]. Passive and total tensions at L_O were observed at baseline tension and maximum tension developed (in response to 10^{-4} M ACH challenge), respectively. Active tension at L_O was calculated as the difference between the total and passive tensions.

Muscle strip wet weights were taken at the end of each experiment. This was accomplished by cutting the portion of muscle that lies between the 4.0 silk points of attachment, briefly blotting on filter paper to remove excess buffer, and weighing on the analytical balance (AND, A & D Co., Ltd-Tokyo, Japan).

Design

Animals were randomly placed in one of the three groups (saline, acute or chronic). Following the treatment period, the distal colon was harvested and prepared for contractility studies. Concentration response curves were constructed to various agonists in the presence or absence of various blockers. (Table III).

TABLE II

INFLAMMATORY EVALUATION CRITERIA

Epithelial Integrity

Score/grade	Description
0	normal
1	space at tips of villi
2	epithelial lifting
3	detached epithelia
4	exposed lamina propria

Vasculature/vasocongestion

Score/grade	Description
0	normal
1	dilation only
2	congestion/dilation
3	severe congestion/dilation
4	frank hemorrhage

TABLE III

MUSCLE CONTRACTURE DESIGN

CRCs	**BATH TREATMENTS***					
NT/AGONIST	NONE	HEX	HEX/A	A	TTX	TTX/A
ACH(10^{-3} - 10^{-9}M)	s/a/c	s/a/c	s/a/c	s/a/c		
NKA(10^{-3} - 10^{-9}M)	s/a/c			s/a/c	s/a	s/a
β-Ala(10^{-6} - 10^{-10}M)	s/a			s/a	s/a	s/a
SP(10^{-6} - 10^{-10}M)	s/a/c			s/a/c	s/a	s/a
Sar-P(10^{-6}- 10^{-10}M)	s/a			s/a	s/a	s/a
Senk(10^{-5} - 10^{-10}M)	s/a			s/a	s/a	s/a
KCl(3 - 120 mM)	s/a/c					

Inflammatory groups tested (s=saline, a=acute, c=chronic).

* Final bath concentrations for treatments are as follows: HEX (Hexamethonium)=10^{-4} M, A (Atropine)=10^{-6}M, TTX (tetrodotoxin)=10^{-6} M.

Muscle Contractility Measurements

Mucosa-free muscle segments, approximately 1.0 x 0.4 cm, were cut and hung in the circumferential axis with 4.0 silk in 7.5 mL organ baths. Tissue was attached to an isometric Grass FTO3 force-displacement transducer and maintained in oxygenated Krebs solution at 37 ^{o} C. Tension changes were calibrated using a 2 g weight and responses were recorded using a Grass model 79 Polygraph (Grass Instruments, Quincy, MA). L_o was determined for all muscle strips using the method described by Percy et. al. and then allowed to equilibrate for a period of 1/2 hr. [44]. Bath treatments were allowed to equilibrate with the tissue for twenty minutes prior to challenge with agonists. Agonists were added to the tissue and tension changes observed for approximately one minute before flushing with new buffer. Tissue was allowed to recover for either 10-15 minutes (ACH and KCl) or 30 minutes (TKs and TK receptor agonists) following each challenge. Old buffer was replaced with newly oxygenated Krebs buffer every 10 minutes and again after every challenge. Bath treatments (see table III) were added back to the appropriate baths after each exchange of new buffer. At the end of the experiment, each muscle strip was exposed to a single concentration of 60 mM KCl (final bath concentration) as an assessment of viability.

Reagents

All chemicals, with the exception of TKs and their specific agonists, were purchased from Sigma (St. Louis, MO). TNBS was purchased from Sigma as a 5% (W/V) aqueous solution of the hydrated acid form. TKs were purchased from Peninsula (Belmont, CA) and the TK receptor agonists from Research Biochemicals International (RBI, Natick, MA). Krebs buffer contained (in mM) 118.5 NaCl, 4.75 KCl, 2.54 $CaCl_2$, 1.19 $MgSO_4$, 25 $NaHCO_3$, 1.19 NaH_2PO_4 and 11.0 glucose. TKs and receptor agonists were dissolved in 0.01 M acetic acid and stored as stock solutions of 10^{-4} M or 10^{-3} M (Senktide) at -80 ^{o} C. The appropriate dilutions from stock were

made in 0.01 M acetic acid on the day of the experiment. Stock solutions of ACH chloride (10^{-1} M) in water were made and stored at 4 ^{o} C. The appropriate dilutions were made in water the day of the experiment. A saturated KCl stock solution (4 M) was made and maintained at room temperature, and appropriate dilutions made the day of the experiment. Working concentrations of atropine sulfate (10^{-3} M), tetrodotoxin (10^{-3} M), and hexamethonium (10^{-2} M) were made fresh weekly and kept at 4^{o} C at all times. Atropine and hexamethonium were dissolved in deionized water, while tetrodotoxin was prepared in a citrate buffer (50 mM citric acid, 48 mM NaH_2PO_4).

Calculation of results

Various standards have been used to normalize tension including challenge to ACH or KCl (% maximal response), or surface area of the muscle strip[19, 44, 45]. Normalization is necessary to correct for the amount of tissue generating the contraction, ensuring accurate comparison among preparations. It is important to normalize to a factor that doesn't change during the inflammatory process, so that only the numerator value of tension developed is considered in the comparison between inflamed groups. There were potential effects of inflammation on KCl and ACH responses. Therefore, tension was normalized to cross-sectional surface area of the muscle. Theoretically, any surface area changes among the groups should generate the same degree of muscle tension development in response to a given agonist. Here, the assumption is that muscle tension development is directly proportional to muscle surface area, and this is constant.

Therefore, the magnitude of isometric tension development was expressed as force per cross sectional area using the following formula[44]:

$$\frac{\text{Force}}{\text{Area}} = \frac{\text{grams tension developed x G (9.8 m/sec}^2\text{)}}{\text{mass (gm wet weight)/\{density (g/mL x length (cm)\}}}$$

L_O and 1.05 g/mL are used for length and muscle density, respectively, in the calculation[21].

Effective concentrations at which 50% of the maximal tension (EC $_{50}$s), as well as maximal tension (T_{max}) develops in response to an agonist, were calculated using Graphpad software program (Graphpad, Inc., Philadelphia, PA).

Statistics

Results were summarized as means $\pm$ SE and unless otherwise noted, concentration response curves were based upon data obtained from 5-8 animals. Analysis of variance followed by a Bonferoni t-test was used to assess singular measurements between controls and inflamed groups (Primer of Biostatics, McGraw-Hill, NY, NY). Concentration response curves expressed as force/cross sectional surface area were compared between control and inflamed groups, using a multivariate analysis of variance (MANOVA) with repeated measures, followed by a t-test designed for comparison of multiple means (Systat 5.2, Systat Inc.).

RESULTS

Histology, Inflammation Characteristics, and Muscle Parameters

Histology and inflammation characteristics

TNBS inflammation significantly increased vasocongestion and compromised epithelial integrity (Table IV). Inflammation scores in both the acute and chronic groups were significantly increased over the saline group ($P < 0.05$). Microscopic measurements of tissue components in acute colon showed no significant changes from saline rats (Table IV). Chronic rats exhibited significantly greater morphologic alterations over saline animals in submucosa, and in both total and circular muscularis externa ($P < 0.05$, Table IV). Additionally, chronic animals had significantly thicker circular muscularis externa than did acute animals ($P < 0.05$). The thickness of muscularis mucosae was unchanged among the groups.

Photomicrographs of distal colon obtained from representative animals in saline, acute and chronic treatment groups are shown in Figures 2 and 3. Saline animals exhibited normal epithelial and muscle architecture. Acute inflammation was characterized by inflammatory neutrophil infiltrate and vasocongestion in the submucosa and mucosa, and damaged epithelia. The chronic tissue showed similar changes in both the mucosa and submucosa, a prominent increase in the muscularis externa thickness. This change appeared to be equal in both circular and longitudinal muscle layers, as the ratio of circular to total was unchanged by chronic inflammation (Table IV). The second major feature of chronic animals was the presence of lymphocytic rather than neutrophilic infiltrate (Figure 3).

Muscle parameters

Physical characteristics of the muscle from inflamed and saline treated groups are summarized in Table V. The length at which maximal contraction was elicited (L_O) did not change in either acute or chronic inflammation. Both wet weight and tension were

TABLE IV

INFLAMMATORY SCORES AND HISTOLOGY MEASUREMENTS

INFLAMMATORY SCORES

	Integrity	Vasocongestion
SALINE (7)	0.8(±0.4)	0.6(±0.2)
ACUTE(7)	2.6(±0.3)*	2.5(±0.2)*
CHRONIC(5)	2.9(±0.8)*	2.2(±0.5)*

Integrity scores are from the epithelium comprising the mucosa, while vasocongestion scores represent the average of lamina propria, submucosa and muscle layer scores (For rating criteria, see Table II).

HISTOLOGY MEASUREMENTS (μm)

	Sub-mucosa	Muscularis mucosae	Muscularis externa		
			Total	Circular	Ratio (%)
Saline (7)	1136 ± 240	567 ± 76	2227 ± 144	1637 ± 98	73.7 ± 1.5
Acute (7)	1955 ± 161	530 ± 60	1531 ± 162	1120 ± 132	72.8 ± 3.5
Chronic(6)	*4322 ± 1485	1094 ± 354	#*4215 ± 561	#*2878 ± 456	68.4 ± 5.9

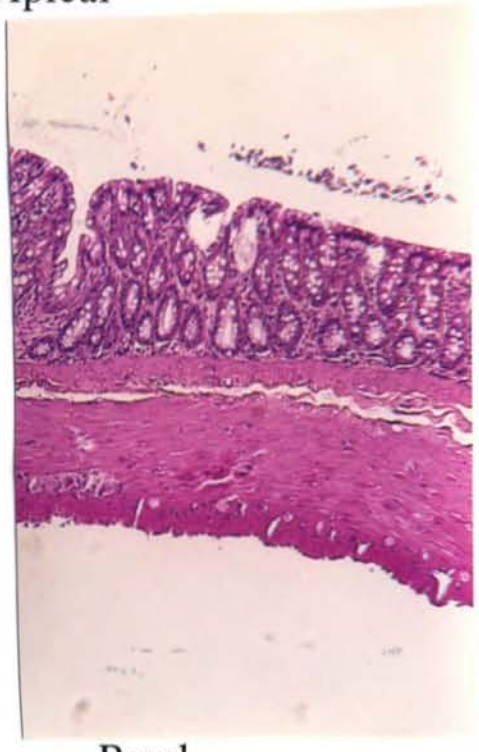
* Indicates results differ significantly from Saline group ($P < 0.05$).

\# Indicates results differ significantly from Acute group ($p < 0.05$).

Figure 2 Photomicrographs of H&E stained tissue sections of distal colon from normal, acutely inflamed, and chronically inflammed male Sprague Dawley rats. Magnification X 58.

Figure 2

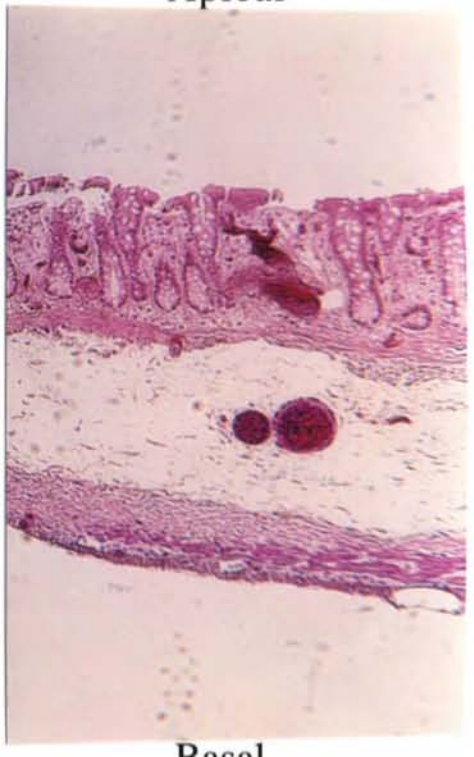
Apical



Basal

A. SALINE

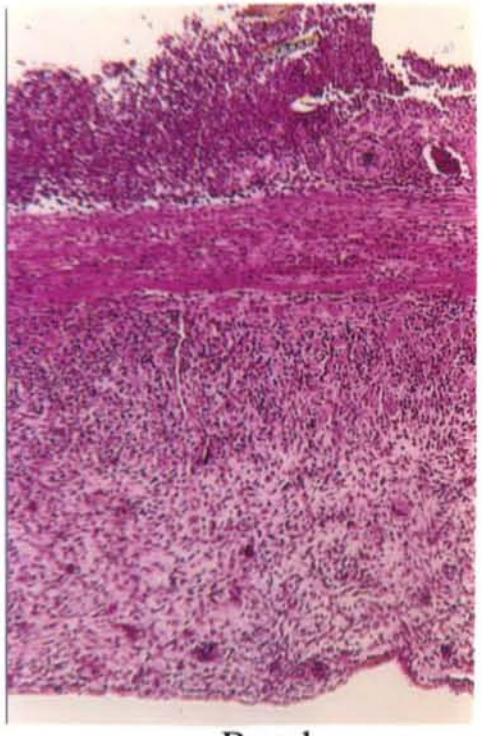
Apical



Basal

B. ACUTE

Apical

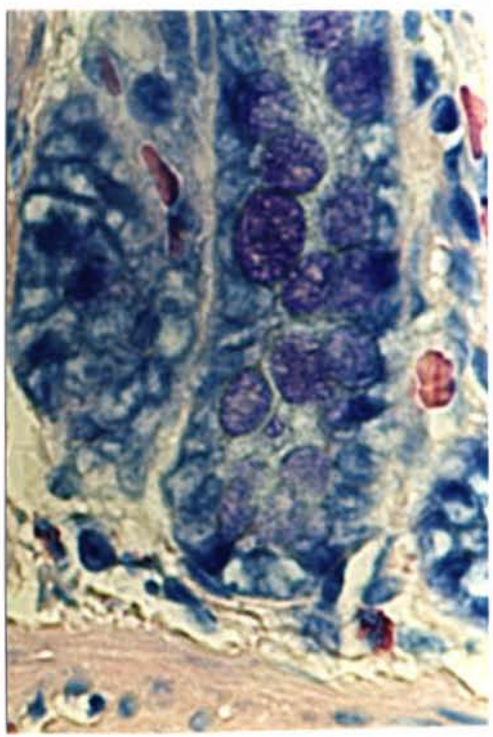


Basal

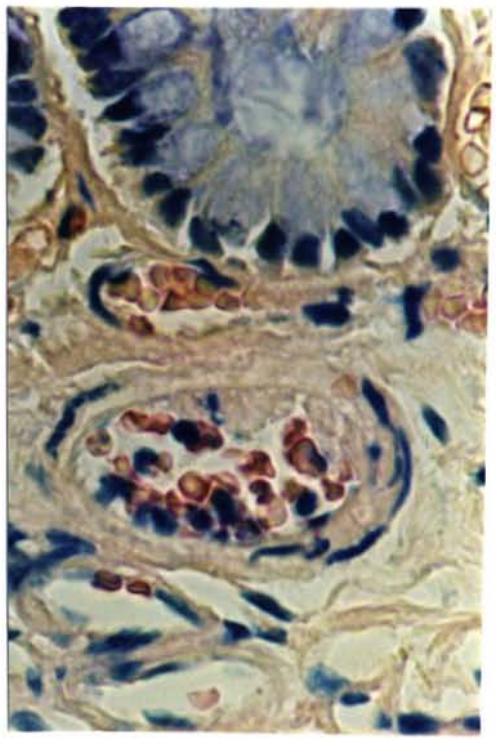
C. CHRONIC

Figure 3. Photomicrographs of Giemsa stained tissue sections of distal colon from normal, acutely inflamed, and chronically inflamed male Sprague Dawley rats. A and B show areas from base of villus crypt near muscularis mucosa. C shows submucosa area only. Magnification X 576.

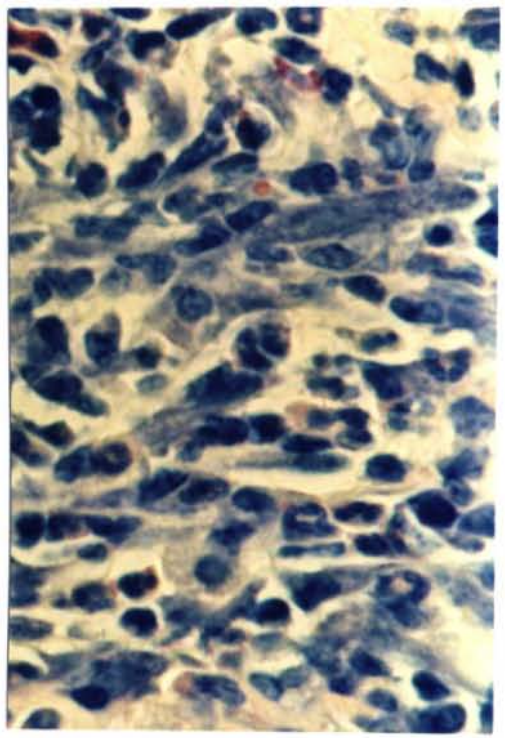
Figure 3



A. SALINE



B. ACUTE



C. CHRONIC

unaffected by acute inflammation. Chronic inflammation, on the other hand, produced significantly increased wet weights over both saline and acute groups ($P< 0.05$). Chronic animals also exhibited a lower passive tension (tension on the muscle at L_o before the contractile event) than the saline treated control group ($P< 0.05$). In addition, total tension in the chronic group was significantly decreased when compared to the acute group ($P< 0.05$).

TNBS induces obvious inflammation documented by characteristic histological changes that occur over the time course of the inflammation. Table VI summarizes these changes observed over time in our rat model of colitis.

TABLE V

PHYSICAL CHARACTERISTICS OF MUSCLE TISSUE

SURFACE AREA PARAMETERS

	L_o (cm)	Wet Weight (mg)
Saline	1.28(±0.05)8	2.6(±0.2)8
Acute	1.19(±0.06)10	2.5(±0.2)10
Chronic	1.19(±0.05)7	#*9.8(±2.4)7

TENSION MEASUREMENTS

	Passive Tension[1]	Active Tension[2]	Total Tension[3]
Saline	9709(±1830)9	8379(±702)9	18087(±1801)9
Acute	7620(±1281)12	12701(±2258)12	20316(±2579)12
Chronic	*3398(±1573)8	6379(±2258)8	#9680(±3285)8

1. Tension within the muscle at L_o before the contraction.
2. Tension developed in reponse to stimulation by ACH (10^{-4} M).
3. The total of passive and active tension developed to ACH (10^{-4} M) challenge.

* Indicates results differ significantly from Control group ($P < 0.05$).

\# Indicates results differ significantly from Acute group ($P < 0.05$).

TABLE VI

SUMMARY OF TNBS INFLAMMATORY COLITIS RAT MODEL

	SALINE	ACUTE	CHRONIC
TREATMENT:	1 mL 0.85% NaCl rectally	1 mL-25 mg TNBS in 50% EtOH rectally	1 mL-25 mg TNBS in 50% EtOH rectally
TIME:	4 hours	4 hours	7 days
HISTOLOGY:	Normal epithelial architecture	Epithelial cell detachment	Occasional intact cells, with more frequent areas of detachment
	Negligible leukocyte infiltration	Neutrophil infiltration (l. propria and s. mucosa)	L. propria, s. mucosa and mucosal lymphocytic infiltration
	Vasocongestion or edema negligible	Prominent vasocongestion	Mild vasocongestion
	Normal muscle architecture	Normal muscle architecture	Thickening of muscularis externa

Muscle Contraction

INVESTIGATIONS OF SALINE, ACUTE, AND CHRONIC RESPONSES

Stage dependent changes

TNBS inflammation induced progressive changes in the circular smooth muscle response to all the neurotransmitters tested, as well as to depolarizing stimulus with K. Figure 4 a-d compares the response curves of NKA, SP, ACH and K from saline, acute and chronic tissues. Table VII compares maximal tension generated (T_{max}) and effective concentrations at which 50% of the maximal responses were attained (EC_{50} values) from the same experiments. Acutely inflamed tissue responses were significantly different from both saline and chronics. SP and ACH response curves were significantly increased during acute inflammation ($P<0.05$)(Figure 4 a-b). In contrast, the response curve to NKA in acute inflammation was significantly decreased compared to saline tissue ($P<0.02$)(Figure 4 c). All of these changes during acute inflammation took place without sensitivity changes, as the EC_{50}s were comparable to saline values (Table VII). While response curves to K challenge were significantly increased during acute inflammation ($P<0.02$), T_{max} values were not statistically different from saline (Figure 4 d, Table VII respectively). However, EC_{50} of acute tissue K challenges were significantly lower, indicating acute tissue was more sensitive to K depolarization.

Chronic inflammation induced drastic reductions in the response curves compared to the saline treatment (all $P<0.02$, Figure 4 a-d). EC_{50}s and T_{max} from chronic tissues were not calculated and included in Table VII for receptor mediated agonists, because the responses did not reach a maximum (T_{max}). NKA, SP and ACH appeared to show an increase in the EC_{50}s, indicating a decreased sensitivity to these transmitters during chronic inflammation. Chronic tissue challenged with K depolarizations showed a significantly decreased T_{max} value compared to saline tissue, but similar EC_{50}s, suggesting that the sensitivity of

Figure 4. Concentration response curves showing stage-dependent smooth muscle contractions to SP, NKA, ACH, and K in untreated baths. Results are mean ± SE. (ACH saline n=9, ACH acute n=10) * P vs. saline. ● Saline ■ Acute ▲ Chronic

STAGE DEPENDENT-CHANGES IN CONTRACTILE RESPONSE

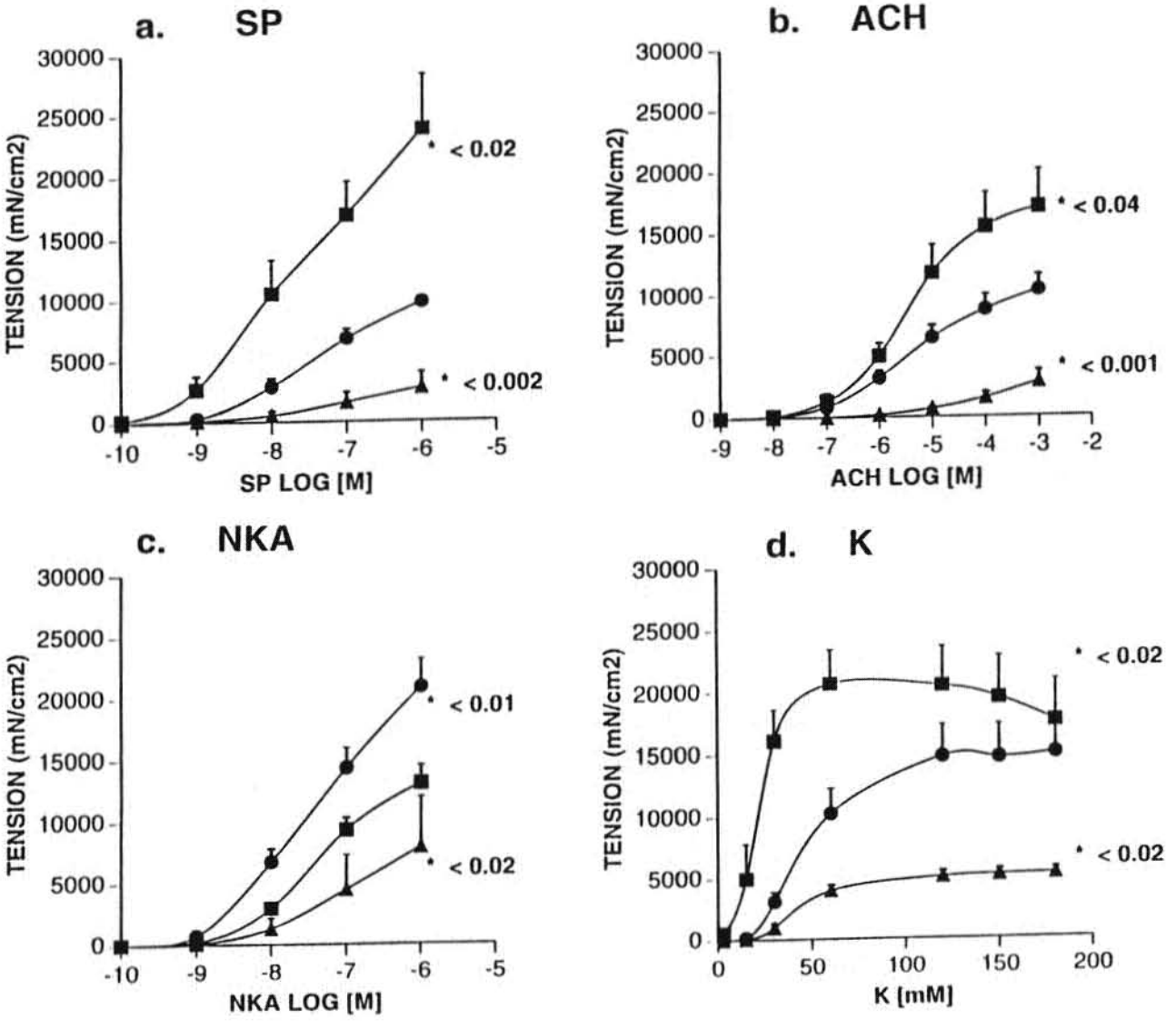


Table VII

Maximum Tensions and EC_{50} Values for Saline, Acute AND Chronic Groups

	SALINE		ACUTE		CHRONIC	
	Tmax	**EC_{50}**	**Tmax**	**EC_{50}**	**Tmax ***	**EC_{50}**
NKA	20860 ± 2270(5)	44.1 nM	13111 ± 1449(7)	43.5 nM	7927 ± 4098(7)	*****
NKA + A	13086 ± 2689(5)	60.1 nM	21412 ± 3246(8)	29.1 nM	3739 ± 1781(6)	*****
SP	9696 ± 452(6)	45.9 nM	23743 ± 4518(8)	32.3 nM	2659 ± 1271(6)	*****
SP + A	18530 ± 2394(6)	27.6 nM	22206 ± 4961(6)	9.2 nM	4608 ± 1933(6)	*****
ACH	10243 ± 1304(9)	4.9 µM	16976 ± 3031(10)	3.5 µM	2751 ± 906(6)	*****
ACH + HEX	25089 ± 2635(6)	9.5 µM	21413 ± 4122(6)	5.1 µM	1167 ± 134(5)	*****
Potassium	14956 ± 2541(6)	45 mM	20625 ± 2732(5)	21 mM	5247 ± 437(5)	45 mM

* Tmax are actual results, not calculated values.
**Refer to results for significant comparisons.

chronically inflamed tissue to K depolarization remained unchanged, while the ability to mount a full response was impaired. Typical responses to 60 mM KCl (as recorded from Grass recorder) are shown for each treatment group in Figure 5.

Cholinergic Influence of TK Response

The data indicate that responses to ACH were altered during inflammation. Since ACH can influence TK responses, tissues from the three groups (saline, acute and chronic) were challenged with TKs in the presence of atropine, which effectively blocks muscarinic mediated ACH responses. Figure 6 a-d shows the response curves to NKA and SP in the presence and absence of atropine in both saline and acutely inflamed tissue. In saline treated tissue, SP response was increased significantly in the presence of atropine, suggesting that SP action was normally inhibited by muscarinic mechanisms in control tissue ($P<0.01$, Figure 6 a). This cholinergic inhibition of SP was lost in acute inflammation, as atropine did not alter the response (Figure 6 b).

A different pattern was seen for smooth muscle response to NKA. In saline-treated tissue, the response to NKA was reduced by atropine. ($P<0.02$, Figure 6 c). Acute inflammation changed this pattern of cholinergic synergism to one of inhibition, as atropine increased the acutely inflamed muscle response to NKA ($P<0.04$, Figure 6 d). Atropine did not alter the sensitivity of the muscle to NKA or SP in either saline or acute tissue, as less than log-fold changes in EC_{50}s were observed (refer to respective EC_{50} values, Table VII).

During the process of chronic inflammation, the muscarinic influence of TK responses was apparently lost. No significant differences in T_{max} or response curves were observed for either SP or NKA responses in the presence or absence of atropine (Table VII, Figure 7 a-b, respectively).

Comparing all three treatment group response curves to both SP or NKA in the presence of atropine, only the chronic treatment was significantly different from saline ($P<0.001$, $P<0.002$, respectively, Figure 8 a-b). Therefore, while the acute response

changes were dependent on muscarinic mechanisms, the alterations in the contractile responses during chronic inflammation occurred independent of muscarinic influence.

Figure 5. Grass recorder tracings showing typical responses from saline, acute and chronic muscle to a 60 mM KCl depolarization. Y axis is grams of tension (scale given) and x axis is time (for all tracings = 25 mm/minute).

REPRESENTATIVE GRASS RECORDINGS FROM TISSUE CHALLENGED WITH 60 mM KCL

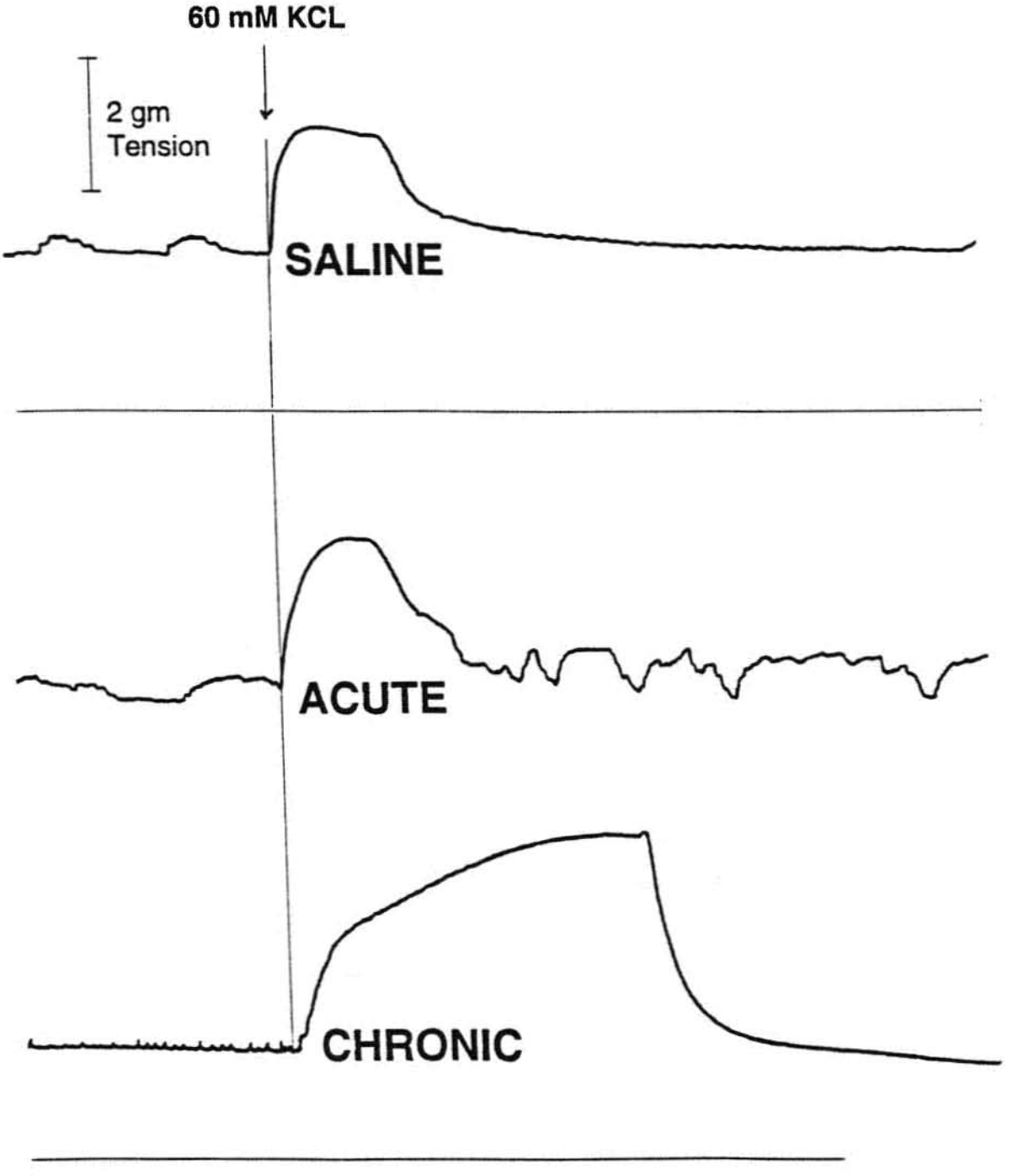


Figure 6. TK concentration response curves in the presence of atropine using muscle from saline treated and acutely inflamed distal colon. Results are mean ± SE. * P vs. untreated bath responses.
● Saline in untreated bath ○ Saline in atropine ■ Acute in untreated bath □ Acute in atropine bath

SALINE AND ACUTE ATROPINE DEPENDENT TK RESPONSES

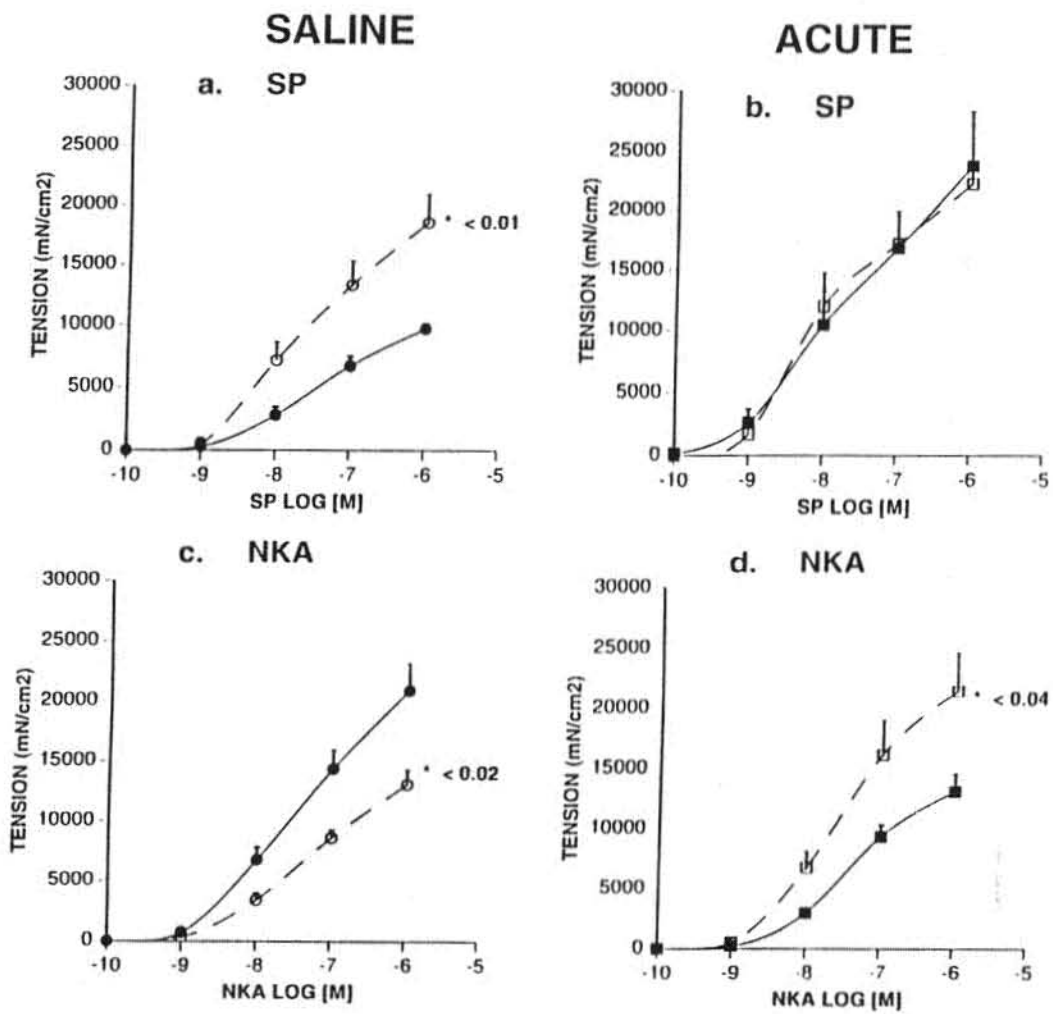


Figure 7. TK concentration response curves in the presence of atropine using muscle from chronically inflamed distal colon. Results are mean ± SE. ▲ Chronic in untreated bath △ Chronic in atropine bath.

CHRONIC ATROPINE-DEPENDENT TK RESPONSES

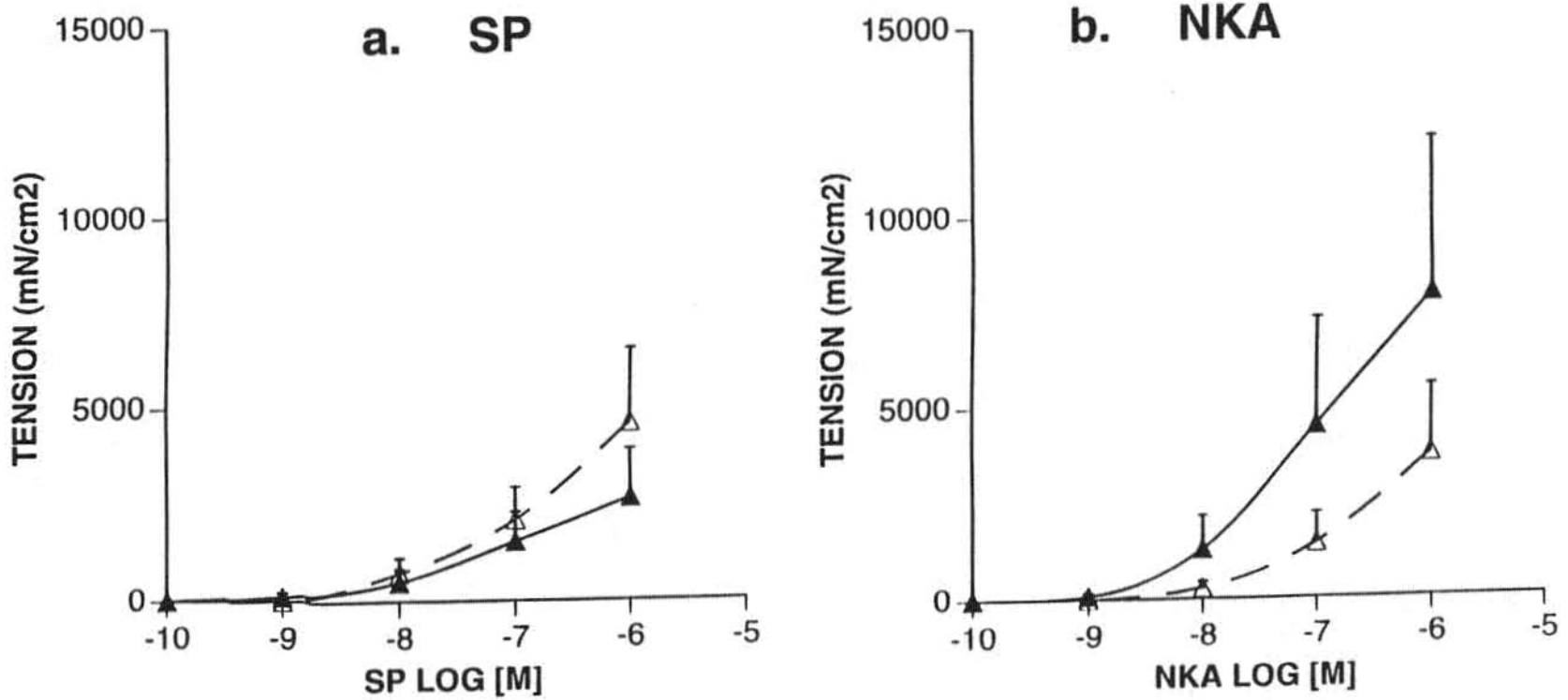
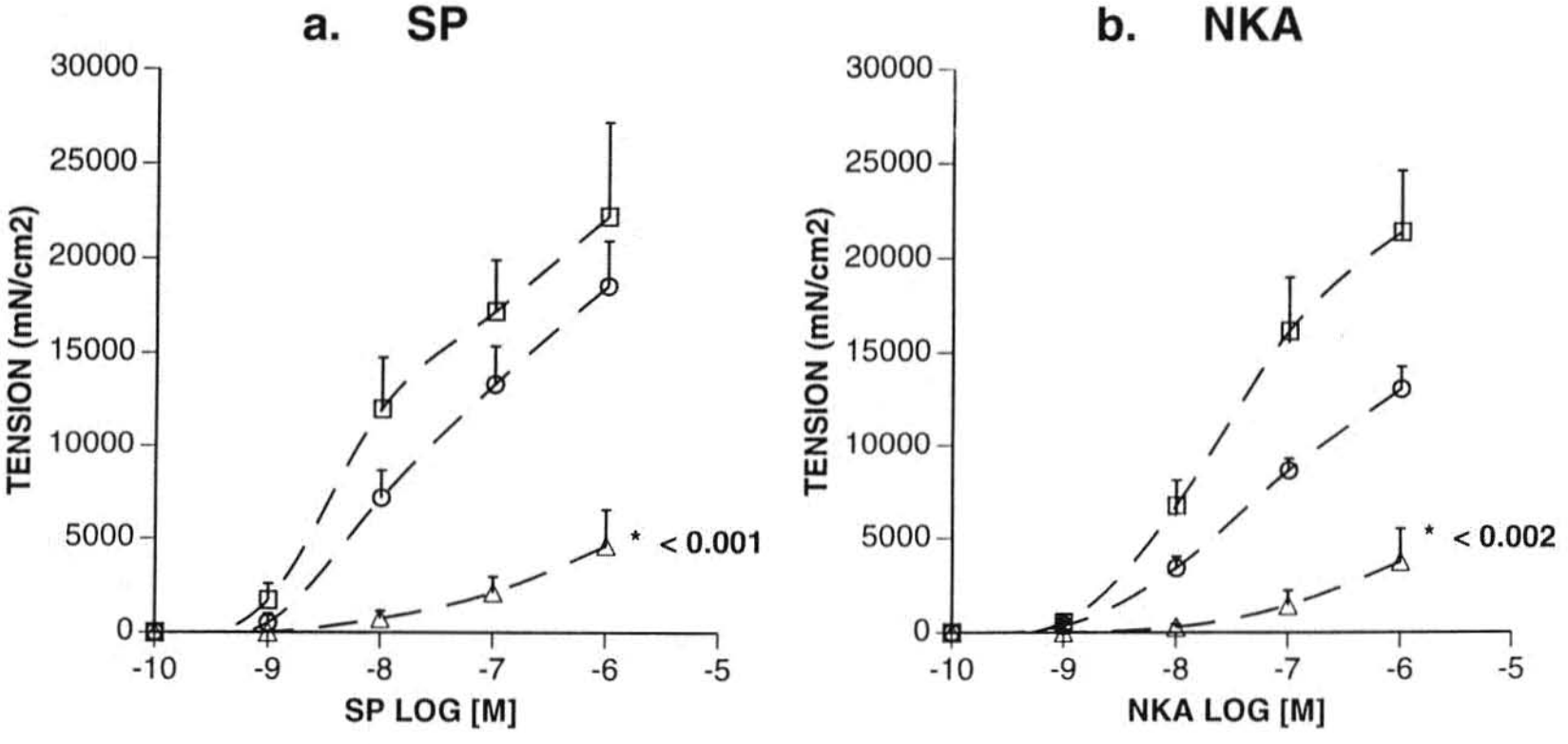


Figure 8. Stage-dependent changes in SP and NKA concentration response curves in the presence of atropine. Results are mean ± SE. * P vs. saline treated animals.
○ Saline in atropine □ Acute in atropine △ Chronic in atropine.

STAGE-DEPENDENT CHANGES OF TK RESPONSES IN ATROPINE



Changes in Cholinergic Mechanisms

Blocking ACH action significantly modified the response to the TKs during inflammation. To further define the mechanism, response curves to ACH were compared in the presence and absence of atropine alone or with hexamethonium (HEX). HEX provides a nondepolarizing ganglionic block by physically blocking nicotinic regulated ion channels [47]. Therefore, in the presence of HEX, ACH could act only through muscarinic receptors. The results are presented in Figure 9 a-c. Saline tissue response to ACH in HEX is significantly increased over the response without HEX ($P<0.002$, Figure 9 a). This indicates that normally, there is a large amount of nicotine mediated inhibition. In acute tissue, the presence of HEX did not significantly increase the response to ACH, indicating a loss of this normal nicotinic inhibition in acute inflammation. Chronic tissue had depressed responses to ACH that were further decreased by the addition of HEX to the bath ($P=0.054$, Figure 9 c). Comparison of saline and acute tissue in the presence of HEX showed no significant difference between response curves (Figure 9 a-b). In contrast, chronic tissue in HEX generated significantly reduced response curves compared to saline (Figure 9 a and 8 c, $P<0.001$). These data suggest the muscarinic receptors were unchanged during acute inflammation, but they may be impaired by a reduction in number or affinity during chronic inflammation. In addition, EC_{50} of ACH response in HEX was similar to untreated baths, indicating that HEX does not alter the sensitivity of ACH in either acute or saline tissue. Sensitivity changes in chronic tissue could not be assessed.

The presence of atropine blocks muscarinic receptors, leaving only nicotinic receptors responsive to ACH. Atropine drastically reduced the ACH response in both saline and acute tissue, and virtually eliminated the already reduced response of chronic tissue (Figure 9 a-c). Therefore, the major contractile force via ACH action on smooth muscle was through muscarinic mechanisms, with a much smaller contribution from nicotinic receptors. Although not shown, response curves to ACH in baths with both atropine and HEX did not differ from response curves in atropine alone for any treatment

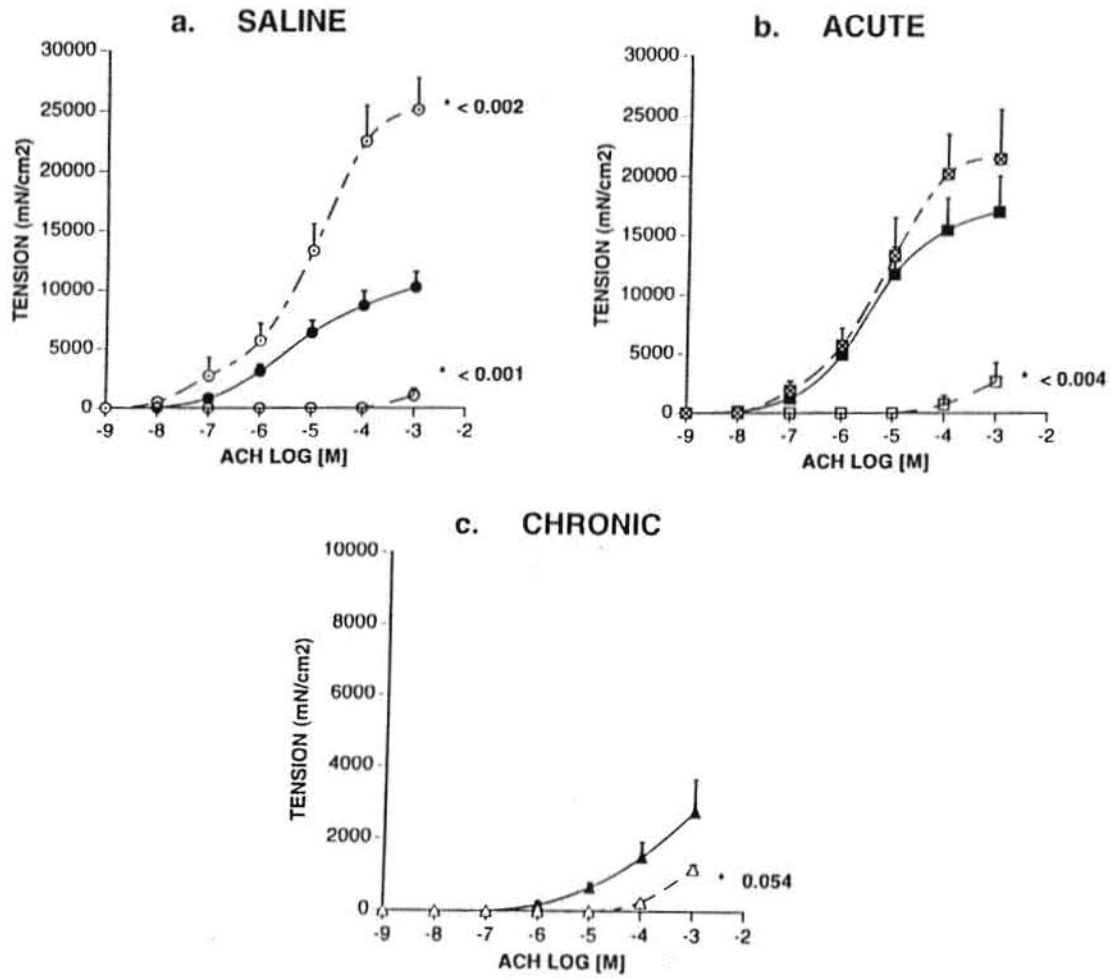
Figure 9. Stage-dependent changes in ACH concentration response curves in the presence and absence of atropine and/or hexamethonium. Results are mean ± SE. (ACH saline untreated n=9, ACH acute untreated n=10), * P vs. untreated bath.

● Saline in untreated bath ⊙ Saline in hexamethonium ○ Saline in atropine.

■ Acute in untreated bath ⊠ Acute in hexamethonium □ Acute in atropine.

▲ Chronic in untreated bath △ Chronic in hexamethonium

STAGE-DEPENDENT CHANGES OF ACH RESPONSES IN ATROPINE AND HEXAMETHONIUM



group (saline, acute or chronic). These results indicate that the major location of muscarinic receptors mediating the contractile response are located on muscle, a point beyond the nicotinic receptors on the enteric ganglia.

To summarize, acute inflammation caused increased responses to SP and ACH, while response to NKA was decreased. Sensitivity to K depolarization was increased, but the maximal tension finally generated was comparable to saline treated rats. Muscarinic inhibition of the response to SP was lossed, while muscarinic regulation of the response to NKA changed from synergism to inhibition. Chronic inflammation caused reduced responses to all neurotransmitters, as well as to K, indicating a general loss of contractile mechanisms. Sensitivities of all neurotransmitters appeared to be decreased in chronics, but K sensitivity remained similar to saline treated animals. Therefore, while there was a decreased ability to attain maximal contraction in response to K and the neurotransmitters, the amount of transmitter necessary to initiate the process was increased, but the amount of K needed for initiation remained unchanged. In addition, chronic tissue showed a total loss of muscarinic influence over both NKA and SP response. Therefore, it is likely that in addition to loss of contractile mechanisms, chronic inflammation decreased receptor activity of both TKs and ACH.

INVESTIGATIONS OF ACUTE RESPONSE CHANGES

Location of Cholinergic Changes of Endogenous TKs

The more intriguing changes of cholinergic influence during acute inflammation were examined further for the location of action by challenging with the TKs under conditions of tetrodotoxin (TTX). TTX effectively blocks sodium-dependent neural excitation. It should be noted that TTX does not block a small amount of neural conduction that is carried by Ca^{+2} current, such as in AH type 2 neurons, and does little to prevent the tonic release of transmitter from the bouton at the level of muscle.

SP

Figure 10 a-c shows SP response curves in saline and acute tissue in the presence or absence of either atropine or TTX. As described previously, atropine increased saline SP response, but did little to further increase the acute SP response (refer to Figure 6 a and b). In the saline group the SP response in TTX was increased over both atropine and untreated tissue (Figure 10 a, $P<0.001$). The T_{max} response in TTX was increased as well, but EC_{50} values stayed the same, indicating no change in sensitivity to SP with the addition of TTX. Response of saline tissue in the presence of TTX and atropine was similar to TTX alone. During acute inflammation, TTX did little to change the SP response compared to untreated acute tissue, as both the response curves, T_{max} and EC_{50} values remained unchanged from untreated acute tissue (Figure 10 b, Tables VII and VIII). It is of interest to note SP response curve comparisons between saline and acute tissue in the presence of TTX. The SP response was significantly decreased during acute inflammation, indicating SP response, exclusive of major neural control, was decreased in acute inflammation ($P<0.003$, Figure 10 c). The T_{max} was also significantly decreased ($P<0.001$), while the EC_{50} remained similar (Table VIII).

NKA

Saline NKA response was previously shown to decrease with atropine, while acute NKA response increased in atropine (Refer to Figure 6 c and 6 d). Figure 11 a-c shows NKA response curve in saline and acute tissue in the presence or absence of either atropine or TTX. Saline NKA response curve in TTX showed a significant increase over untreated saline tissue ($P<0.03$, Figure 11 a). The presence of TTX increased the saline tissue sensitivity to NKA five fold, indicating that neural input normally lessens the sensitivity of muscle to NKA (Table VII and Table VIII). Even in light of these changes, the NKA T_{max} value of saline tissue in TTX was not significantly different from that of untreated saline tissue (Table VII and Table VIII).

Figure 10. SP concentration response curves in saline and acute tissue in the presence and absence of atropine or TTX. Results are mean ± SE. (SP saline TTX, n= 3), * P vs. to Saline untreated bath, # P vs. Saline + TTX.

ʘ Saline in TTX o Saline in atropine ● Saline in untreated bath

⊠ Acute in TTX □ Acute in atropine ■ Acute in untreated bath

TTX-SENSITIVE NEURAL REGULATION OF SP RESPONSE

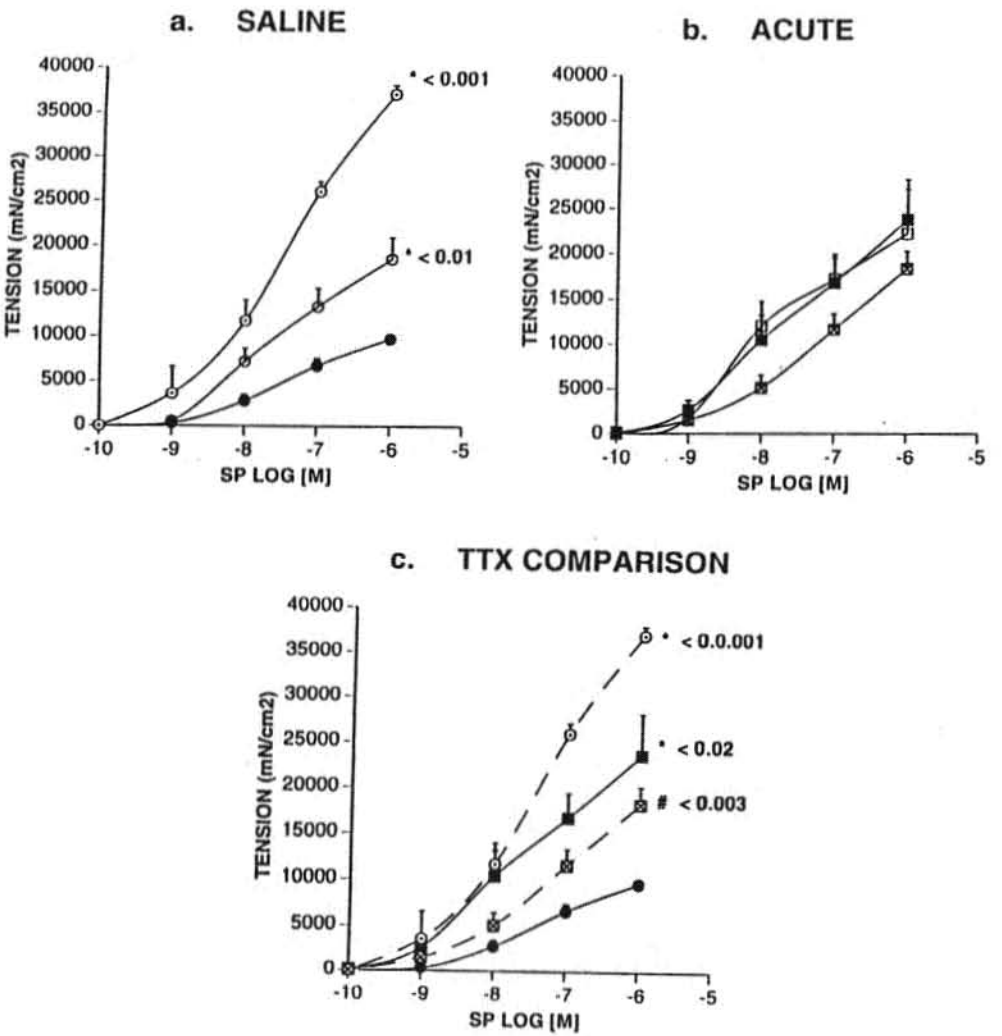


Table VIII
Maximum Tensions and EC_{50} Values for Saline and Acute Groups

	SALINE		ACUTE	
	Tmax	**EC_{50}**	**Tmax**	**EC_{50}**
NKA+TTX	25906 ± 6504(6)	8.2 nM	17503 ± 3044(6)	18.7 nM
NKA+TTX/A	25160 ±1893(7)	11.5 nM	19747 ± 3272(6)	21.4 nM
β-ALA	26434 ± 1055(7)	55.4 n nM	18112 ± 697(6)	122.3 nM
β-ALA+A	29624 ± 569(7)	116.9 nM	18784 ± 51(6)	90.7 nM
β-ALA+TTX	20402 ± 99(3)	28.4 nM	12819 ± 460(3)	126.1 nM
β ALA+TTX/A	18039 ± 85(3)	20.6 nM	12256 ± 555(3)	98.8 nM
SP +TTX	37078 ± 1014(3)	49.4 nM	18330 ± 1946(5)	111.3 nM
SP+TTX/A	34347 ± 3498(5)	21.6 nM	19703 ± 1440(6)	36.7 nM
SAR P	25393 ± 1477(5)	40.0 nM	17772 ± 532(5)	27.9 nM
SAR P+A	22126 ± 759(5)	12.4 nM	21493 ± 711(5)	33.8 nM
SAR P+TTX	18068 ± 290(7)	20.0 nM	12311 ± 60(7)	25.3 nM
SAR P+TTX/A	21843 ± 250(6)	8.8 nM	15199 ± 69(7)	24.3 nM
SENK	3395 ± 8(6)	58.8 nM	642 ± 397(5)	****
SENK+A	2497 ± 49(5)	39.4 nM	366 ± 249(5)	****
SENKTTX	7363 ± 559(7)	814.7 nM	895 ± 405(5)	****
SENK+TTX/A	4622 ± 31(6)	816.4 nM	537 ± 537(5)	****

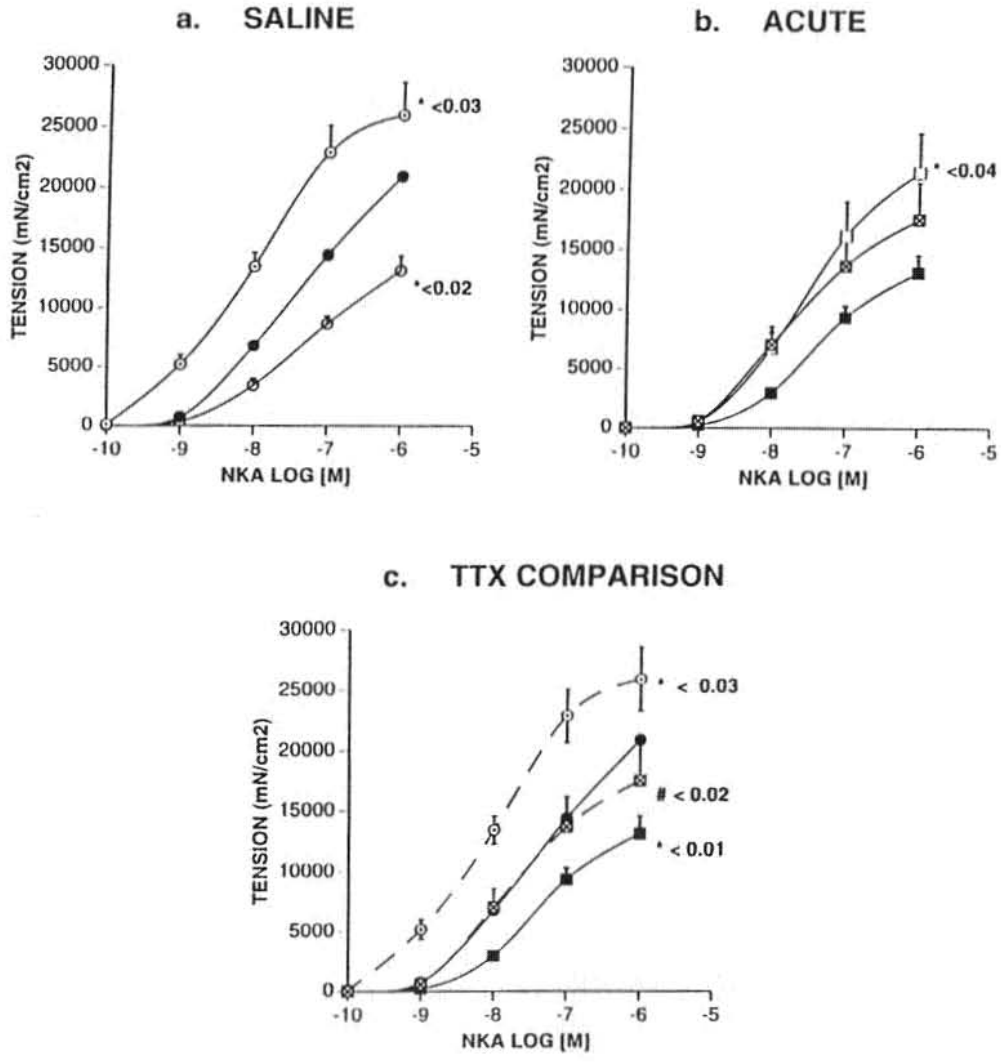
*Refer to results for significant comparisons.

Figure 11. NKA concentration response curves in saline and acute tissue in the presence and absence of atropine or TTX. Results are mean ± SE. * P vs. Saline untreated bath, # P vs. Saline in TTX.

● Saline in untreated bath ⊙ Saline in TTX ○ Saline in atropine

■ Acute in untreated bath ⊠ Acute in TTX □ Acute in atropine.

TTX-SENSITIVE NEURAL REGULATION OF NKA RESPONSE



In acute tissue, the presence of TTX did not significantly change the NKA response curve from untreated acute tissue (Figure 11 b, P=0.082). Furthermore, the presence of TTX during acute tissue challenge elicited no significant changes in T_{max} or EC_{50} values. (Tables VII and VIII). Again the interesting comparison between saline and acute tissue response to NKA in the presence of TTX shows similarity to that of SP. The NKA acute response curve in the presence of TTX was significantly decreased compared to the saline response under the same bath treatment ($P<0.02$, Figure 11 c). This occurred without statistically different T_{max} values and less than log-fold differences in EC_{50}s values (Table VIII).

Receptor Specific TK Agonists

Acute inflammation caused muscarinic dependent changes in the endogeneous TKs, NKA and SP. SP and NKA work primarily through NK_1 and NK_2 receptors respectively, but both exhibit some crossreactivity for the NK_3 receptor. NK_3 affinity is highest for Neurokinin B (NKB), which is present in the brain, but not in the gut. Therefore, SP and NKA may act at any of the three receptors. Alteration of receptor activity may explain TK responses seen during acute inflammation. To investigate changes in receptor activity, saline and acutely inflamed colonic muscle were studied using specific agonists for NK_1, Sar substance P; NK_2, β-alanine; and NK_3, senktide, in the presence and absence of atropine, TTX, or atropine combined with TTX.

NK_1

To investigate the differences between the endogenous SP response and the receptor specific agonist for NK_1, comparisons were made under different bath conditions. Saline tissue comparisons of SP and Sar P responses are illustrated in Figure 12 a and b and in Tables VII and VIII. Both Sar P response curves and T_{max} were significantly higher than SP responses in untreated baths ($P<0.0001$, Figure 12 a and 12 b, Table VII and VIII). The two NK_1 agonists gave similar responses in atropine treated baths (Figure 12 a and 12 b, Tables VII and VIII). In contrast, SP response curves, as well as T_{max} values were significantly greater than Sar P in the presence of TTX ($P<0.002$, Figure 12 a and 12 b, Table VIII). This suggests there was a greater neural inhibition of SP response than of Sar P response in saline treated tissue. All of these changes are independent of sensitivity differences, as the EC_{50} values were similar (Tables VII and VIII). In addition to the already described atropine inhibition, it is apparent that SP activity in saline tissues contained another inhibitory neural component. SP saline response in the presence of TTX was not only significantly increased over the SP untreated bath response, but it was also significantly higher than the response to SP in atropine ($P<0.001$, P,0.005 respectively, Figure 12 a). Unlike SP, Sar P exhibited neither atropine or TTX sensitivity in saline tissue, as the response curves and T_{max} values under these conditions were similar to untreated bath responses (Figure 12 b, Table VIII).

Acute tissue SP and Sar P responses in untreated baths are compared in Figures 12 c and in Tables VII and VIII. In acute tissue, SP response was now comparable to that of Sar P (Figure 12 c). Additionally, acute Sar P response curves were not significantly different from saline Sar P responses (Figure 12 c). Although not shown graphically, the Sar P response in acute tissue was unchanged by the presence of TTX, atropine or the combination of the two (refer to acute EC_{50} and T_{max} values in Table VIII). Therefore, unlike SP, Sar P response was unchanged by acute inflammation and exhibited no atropine or TTX sensitivity in either saline or acute tissue.

To observe the changes in receptor activity at the level of the muscle, saline and inflamed tissue were challenged with the two NK_1 agonists in the presence of TTX (Figure 12 d). In the presence of TTX, Sar P showed significantly decreased acute responses compared to saline tissue in similar bath conditions ($P<0.042$, Figure 12 d). Similarly, SP under TTX bath conditions showed significantly decreased acute responses compared to saline tissue ($P<0.003$, Figure 12 d). Therefore, in the absence of neural influence, both NK_1 agonists had a similarly decreased response at the level of the muscle during acute inflammation.

NK_2

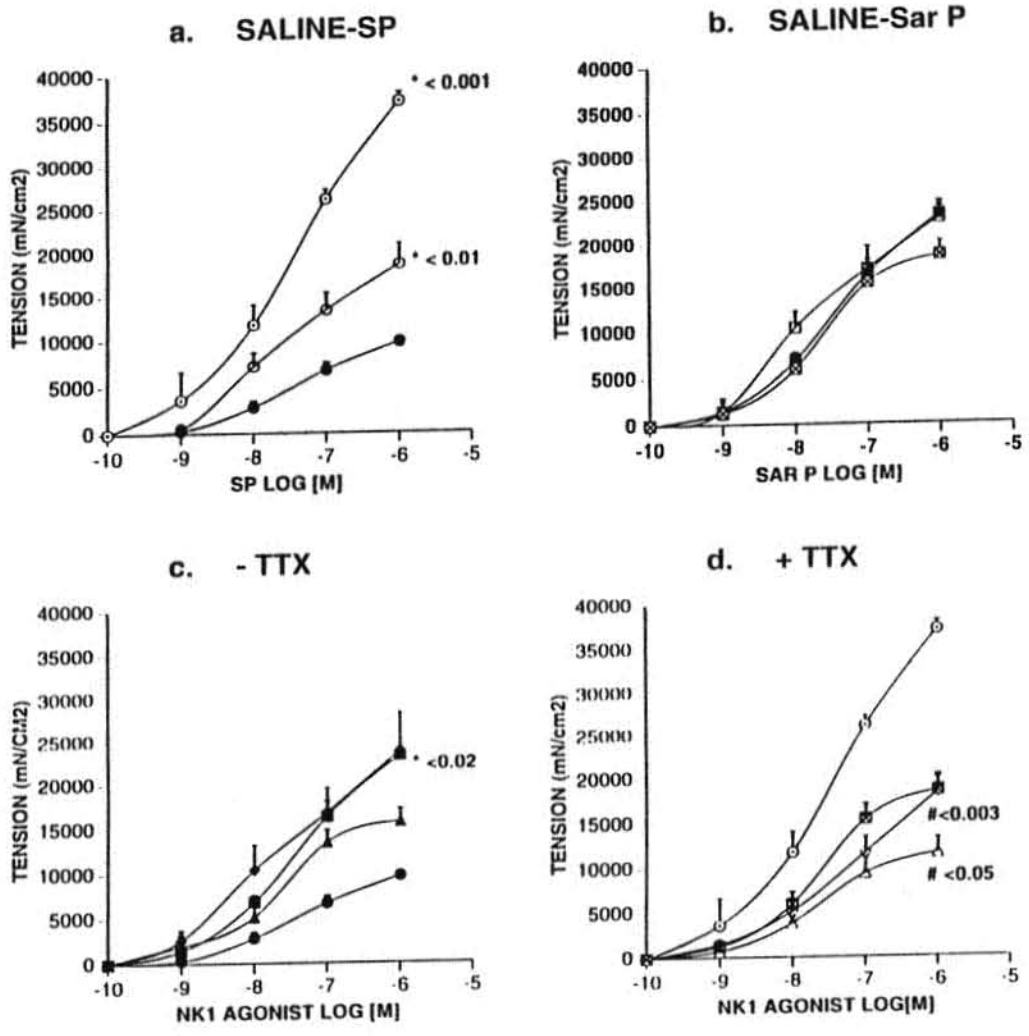
Saline tissue comparisons of NKA and β ALA responses are illustrated in Figure 13 a and 13 b and in Tables VII and VIII. Both NKA and β ALA response curves, EC_{50}s and T_{max} values were similar in untreated baths (Figure 13 a and 13 b, Table VII and VIII). Saline NKA response significantly decreased with atropine conditions and increased with TTX ($P<0.02$, $P<0.03$ respectively, Figure 13 a). Saline NKA response, compared to that of saline β ALA, was lower in the presence of atropine, and significantly higher in the presence of TTX ($P=0.094$, $P<0.045$, respectively). Additionally, in the presence of TTX, the saline NKA response was 5 times more sensitive than in untreated baths (Table VIII). In contrast, saline β ALA response remained unchanged by either atropine or TTX, and no sensitivity changes were apparent. This indicated that in saline tissue, NKA response had both positive and negative modulating components that were not shared by β ALA. Saline β ALA response was neither atropine nor TTX sensitive.

Acute NKA and β ALA responses in untreated baths are compared in Figure 13 c and Tables VII and VIII. In acute tissue, NKA and β ALA response curves were similar, although β ALA had a significantly higher T_{max} ($P<0.02$, Figure 13 c). Additionally, both acute β ALA and NKA response curves were significantly decreased from their respective saline values ($P<0.04$, $P<0.01$, respectively)(Figure 13c). Although not shown graphically, the

Figure 12. NK_1 agonist (Sar P/SP) concentration response curves from saline and acute tissue in the presence and absence of atropine or TTX. Results are mean ± SE. * P vs. SP Saline untreated bath, #P vs. Saline + TTX.

● SP Saline in untreated bath ⊙ SP Saline in TTX ○ SP Saline in atropine
◆ SP Acute untreated bath ◇ SP Acute + TTX
■ Sar P Saline in untreated bath ⊠ Sar P Saline in TTX □ Sar P Saline in atropine
▲ Sar P Acute untreated bath △ Sar P Acute + TTX.

TTX-SENSITIVE NEURAL REGULATION OF NK1 RESPONSE



β ALA response in acute tissue was unchanged by the presence of TTX, atropine or the combination of the two (refer to acute EC_{50} and T_{max} values in Table VIII). Therefore, like NKA, β ALA response was similarly decreased by acute inflammation, but unlike NKA, β ALA exhibited no atropine or TTX sensitivity in either saline or acute tissue.

To observe the changes in receptor activity at the level of the muscle, saline and inflamed tissue were challenged with the NK_2 agonists in the presence of TTX (Figure 13 d). In the presence of TTX, both β ALA and NKA had significantly decreased acute responses compared to saline tissue in similar bath conditions ($P<0.004$, $P<0.02$, respectively)(Figure 13 d). Decreased acute β ALA response may be due to decreased sensitivity, as it was five times less sensitive than the saline tissue (Table VIII). Therefore, while NKA exhibited neural influence not shared by β ALA, in the absence of neural influence, both NK_2 agonists had similarly decreased responses at the level of the muscle during acute inflammation.

NK_3

Acute changes in NK_3 receptor activity are reflected by challenges to senktide and are illustrated in Figure 14 a and c. Senktide responses were nearly a log fold lower than any other agonist, suggesting a low level of NK_3 receptors were present in the distal colon (Figure 14 a and 14 c). Senktide response curves and T_{max} values were significantly decreased during acute inflammation ($P<0.0001$, Figure 14 a and 14 c). Atropine, TTX, or the combination did not change senktide responses in either saline or acute animals (refer to Table VIII). Thus, senktide exhibited no atropine or TTX sensitive neural component, and acute inflammation caused the NK_3 response at the level of the muscle to decrease.

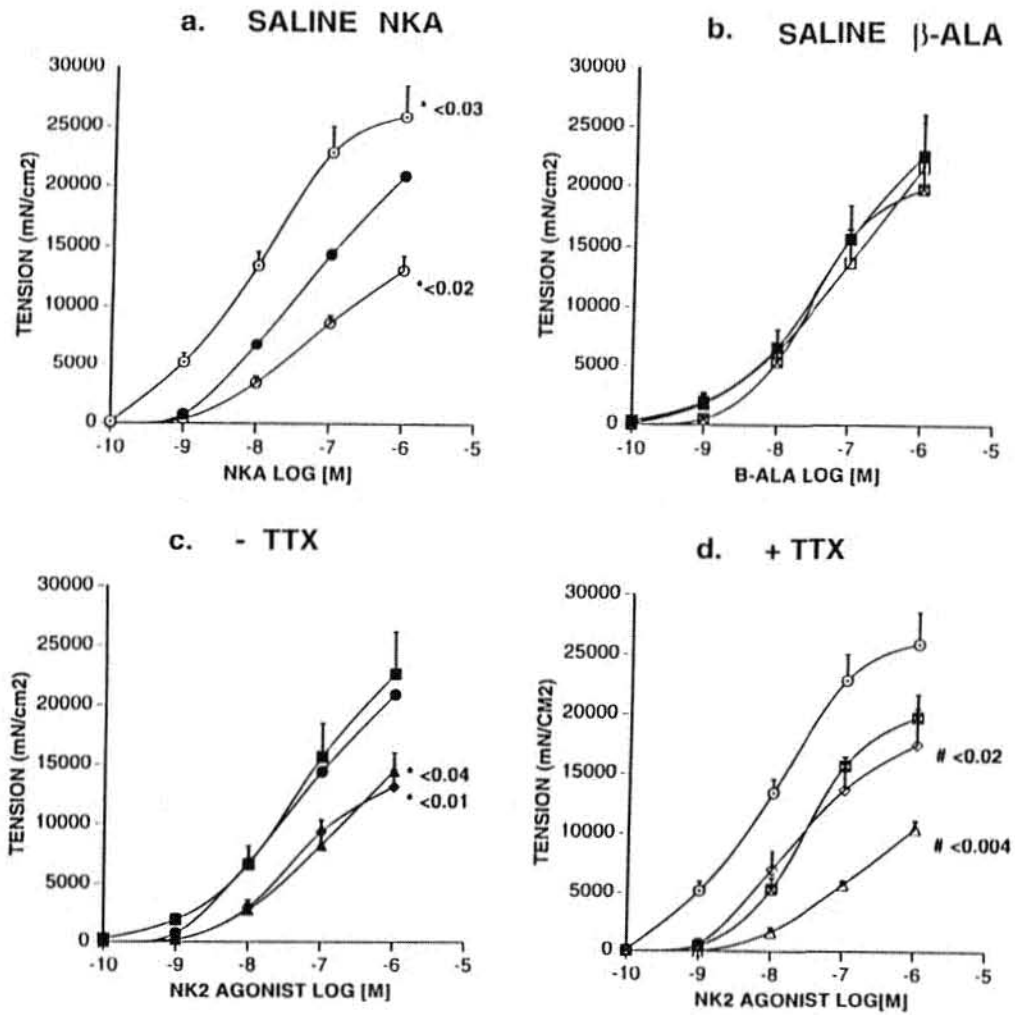
Changes in Relative Responses.

Relative contractile strengths of endogenous TKs and receptor specific agonists were compared in saline or acutely inflamed tissue (Figure 14 a-d). Saline response to NKA was significantly greater

Figure 13. NK_2 agonist (β-Ala/NKA) concentration response curves from saline and acute tissue in the presence and absence of atropine or TTX. Results are mean ± SE. (β-Ala saline and acute + TTX n=3) * P vs. Saline untreated bath, # P vs. Saline + TTX.

● NKA Saline in untreated bath
⊙ NKA Saline in TTX
○ NKA Saline in atropine
◆ NKA Acute in untreated bath
◇ NKA Acute + TTX
■ B-Ala Saline in untreated bath
⊠ B-Ala Saline in TTX
□ B-Ala Saline in atropine
▲ B-Ala Acute in untreated bath
Δ B-Ala Acute + TTX.

TTX-SENSITIVE NEURAL REGULATION OF NK_2 RESPONSE



than that of saline SP response ($P<0.002$, Figure 14 b). In acute inflammation, the pattern was reversed: SP response was now significantly greater than NKA response ($P<0.03$, Figure 14 d). In contrast, β ALA and Sar P produced similar contractile responses whether the comparison was in acute or saline animals (Figure 14 a and 14 c, Table VIII). These results showed that the inflammation induced changes in the response to endogenous TKs were not mimicked by their selective receptor agonist.

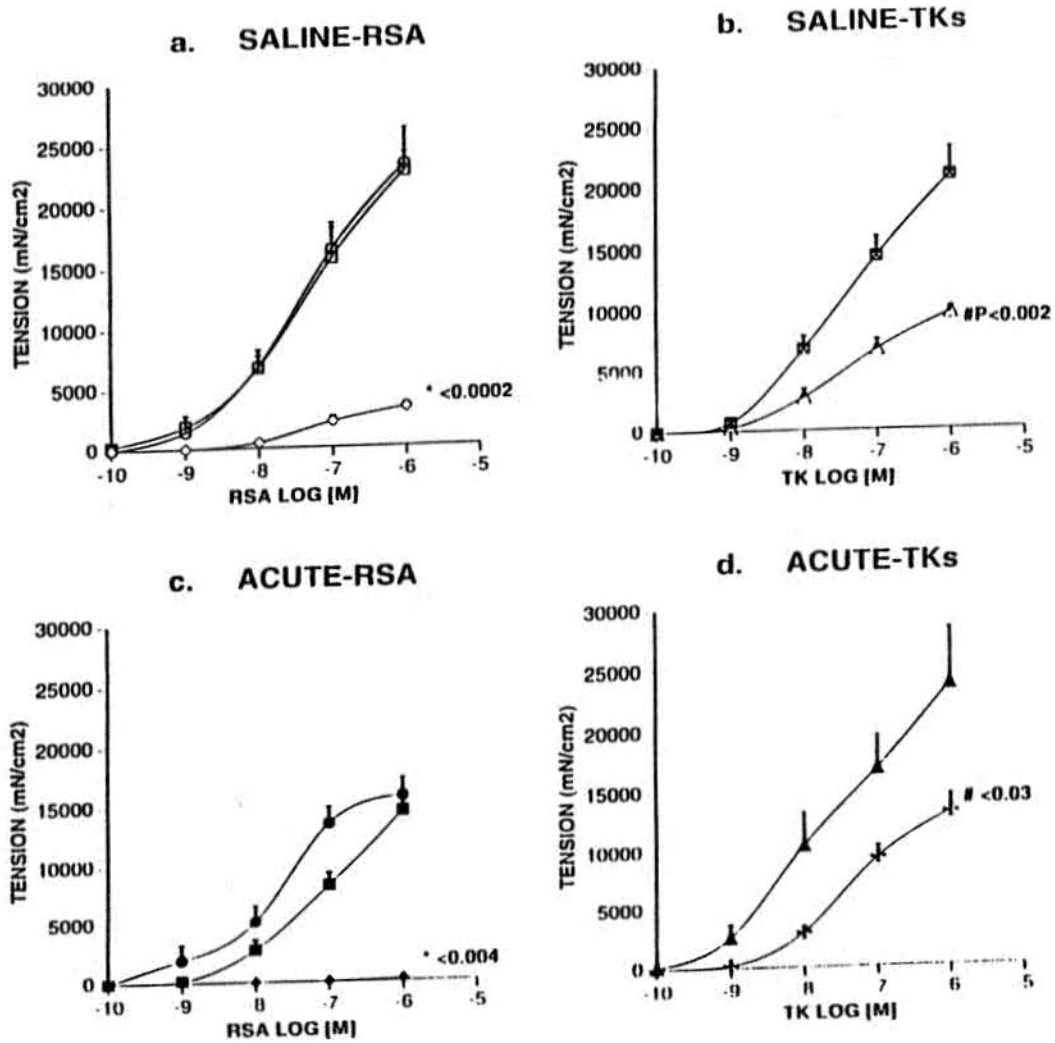
Figure 14. Concentration response curves from saline and acutely inflamed tissues showing relative contractile strengths of receptor specific agonists (RSAs) and tachykinins (TKs). Results are mean ± SE. * P vs. other RSAs, # P vs. other TK.

□ Saline B-Ala ■ Acute B-Ala ◙ Saline NKA ✣ Acute NKA

○ Saline Sar P ● Acute Sar P Δ Saline SP ▲ Acute SP

◇ Saline Senk ◆ Acute Senk

RELATIVE COMPARISONS OF RSA AND TK CONTRACTILE RESPONSE



Summary of Muscle Contraction Results

The results from our acute animal experiments indicate that compared with saline controls, there was an increased contractile response to SP and ACH, with decreased responses to NKA. Sensitivity to K was increased, while the maximal contractile response was unchanged. The action of SP and NKA via atropine-sensitive neurons was altered during acute inflammation. Nicotinic inhibition of the contractile response to ACH was decreased during acute inflammation compared to controls. Receptor-selective agonist experiments indicate that alterations in the action of TKs via atropine sensitive pathways did not occur through changes in any of the three known TK receptors.

Experiments with chronically inflamed colon documented an overall decrease in the ability of the muscle to generate a contraction. The sensitivity of the muscle to all neurotransmitters appeared decreased, while the sensitivity to K-induced depolarization remained unchanged. Additionally, there was a loss of SP and NKA action via atropine sensitive neural pathways and nicotinic inhibition of the response to ACH.

DISCUSSION

Our experiments document changes in the contractile response of colonic smooth muscle to TKs and in their cholinergic modulation, occurring after both acute and chronic inflammation. Significant differences in TK activity were observed between acutely and chronically inflamed colon. The TKs and ACH are important excitatory neurotransmitters participating in normal motility patterns. Both transmitters play integral roles in the neural circuitry of the peristaltic reflex, contributing to the ascending contractile component[23]. In the colon, motility patterns of mixing and mass movement ultimately determine the difference between healthy bowel habits or increased urgency and frequency of defecation associated with forms of IBD.

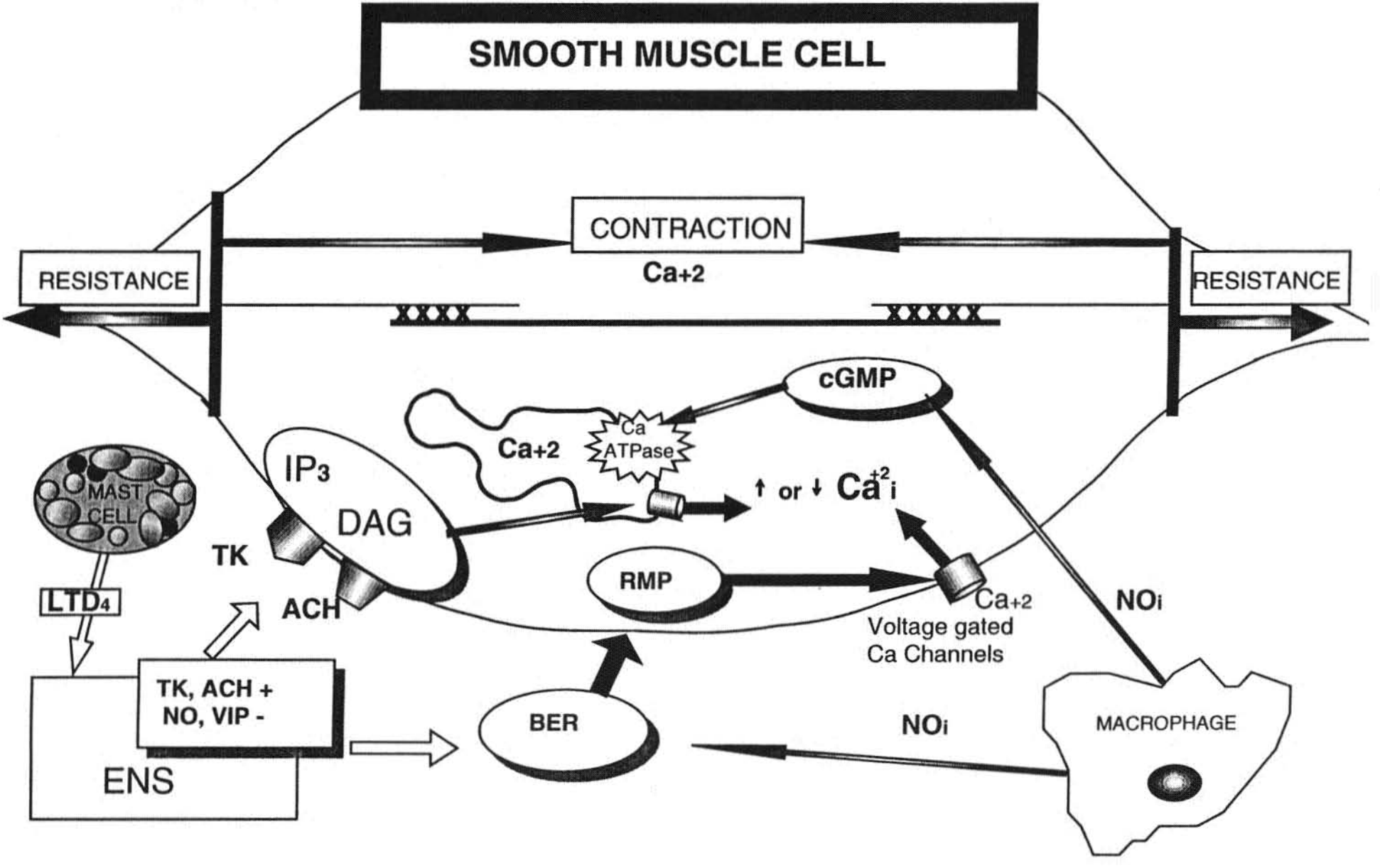
Motility patterns are not only affected by alterations in neurotransmitter profile, but also by smooth muscle contractility, which is a product of neural input and inherent smooth muscle characteristics. Our experiments document that both muscle and nerve alterations contribute to inflammation-induced changes of the smooth muscle contractile response to TK and ACH. It is these abnormal contractile responses that underlie the motility changes characteristic of IBD.

MUSCLE CHANGES

Inherent smooth muscle characteristics contribute to the contractile state by factors illustrated in Figure 15. The combination of resting membrane potential, BER, crossbridge formation between actin and myosin filaments, voltage gated calcium channels, receptor activity, second messenger pathways and resistive properties of the surrounding tissues define the ability of the smooth muscle cell to mount a contractile force. Any of these may be targeted during inflammation. It is important to note that some alterations require changes in protein synthesis that could not have taken place within the time frame of our acute experiments. However, chronic

Figure 15: Smooth muscle cell illustration showing mechanisms that contribute to muscle contractility.

MECHANISMS CONTRIBUTING TO MUSCLE CONTRACTILITY



experiments are well within the time interval required for translational and transcriptional changes necessary for redirected protein synthesis.

Acute Changes

The ability of the muscle to depolarize with K challenge was enhanced in acute inflammation, indicating an increased excitability of the tissue. Although the T_{max} was similar to the non-inflamed state, it was achieved at a lower concentration of K (60 mM vs 120 mM, Figure 4 d) and the depolarization process was initiated at a lower K concentration (EC_{50} = 21 mM vs 45 mM, Table VII). It is therefore, unlikely that muscle contractile mechanisms are altered by acute inflammation. The physical properties of acutely inflamed tissue would support this conclusion, since wet weights, Lo and passive, active and total tensions were similar in the acutely inflamed group and the saline treated group (Table V). Additionally, the time frame required for protein synthesis to redirect these processes was not within the time window of the acute model.

Increased excitability of intestinal smooth muscle has been documented in several models of acute ileitis[16, 17, 57] Excitability was characterized myoelectrically by increases in spike bursts, propagating spike bursts, migrating action potential complexes, repetitive bursts of action potentials, and the percent of slow waves with spikes in two different models of ileitis[57]. In our model of colitis, increased muscle excitability was documented by increased sensitivity to K depolarization. This may have occured through several mechanisms. Neurotransmitter and inflammatory mediator profiles are both altered during the time course of inflammation. Either may act directly on the muscle to increase the resting membrane potential, making the muscle more easily depolarized. Alternatively, the resting membrane potential of the muscle may have been changed via action on the interstitial cells of Cajal (ICC), the pacemaker cells responsible for the slow wave activity, also known as the BER. An increase in either the frequency, amplitude or

duration of slow waves would allow the muscle to depolarize easier to a given stimulus.

In the dog colon, SP and ACH transmitters enhance smooth muscle contractibility by increasing the slow wave plateau phase duration and amplitude[30]. Both SP and ACH have been shown to be altered during inflammation[7, 12]. Electrical field stimulation (EFS) studies of cat colon demonstrate that VIP, an inhibitory neurotransmitter, decreases slow wave frequency [6]. VIP has been shown to be decreased in colon from both ulcerative colitis and Crohn's patients[33, 39]. Additionally, slow wave characteristics may have been altered by loss of nicotinic inhibition during acute inflammation. Nicotine mediated inhibitory neural influence decreases slow wave amplitude, duration and rate of rise[51]. Thus, the observed loss of nicotinic inhibition seen in our acute inflammatory model may have contributed to the excited state by increasing slow wave frequency. Therefore, either an increase in SP and ACH, a decrease in VIP, or the loss of nicotinic inhibition may have influenced slow wave characteristics, contributing to a more excitable muscle.

SP and ACH both tend to depolarize, while VIP hyperpolarizes the muscle resting membrane potential. Therefore, increases in SP and ACH, or decreases in VIP may increase muscle resting membrane potential, making the muscle inherently more excitable. Alternatively, an enhanced opening of voltage gated Ca channels, or uncoupling of second messenger systems such as guanylate cyclase may have impacted on the speed of depolarization, independent of a given resting membrane potential (refer to Figure 15).

Inflammatory mediators, as well as changes in neurotransmitter profile may have contributed to the more excitable muscle state. Histamines, prostaglandins, leukotrienes, and NO are examples of inflammatory mediators released from mast cells and macrophages that may have influenced smooth muscle contractility. LTD4, the 5 lipoxygenase product of arachadonic acid metabolism, is an inflammatory mediator that contributes to the excitable muscle state seen in inflammation. Increased myoelectrical activity characteristic of the inflamed muscle state were all reduced or

completely blocked in the presence of an LTD4 antagonist, indicating a role for LTD4 mediated exictation[57]. Additionally, EFS studies utilizing a LTD4 antagonist, provide evidence that LTD4 increases neurotransmitter release during neural excitation[20]. Therefore, it is likely that LTD4 acts similarly in colitis, increasing muscle excitability through tonic release of excitatory neurotransmitters.

In spite of an enhanced sensitivity of the muscle to K depolarization during acute inflammation, all three TK receptors; NK_1, NK_2, and NK_3 exhibited decreased response at the level of the muscle. Reduction in NK_1 activity (acute Sar P in TTX) occurred independently of sensitivity changes. SP action on muscle is known to undergo tachyphylaxis, a process by which previous exposure to the chemical desensitizes tissue, causing reduced responses on subsequent challenges. In inflammation, NK_1 receptors appear uninfluenced by the process of tachyphylaxis, as the EC_{50}s remained similar between saline and acute groups. Therefore, the reduced NK_1 activity at the muscle may have resulted from either a decreased efficiency of the receptor coupling with second messenger pathways, or attenuation of the second messenger signal. In contrast, decreased NK_2 (acute β ALA in TTX) and NK_3 responses (acute Senk in TTX) indicate sensitivity changes in the receptor (Table VIII). These data support a decreased sensitivity and response via NK_2 receptors, and probably NK_3 receptors. This may have resulted from either a reduction in the receptor number, by a process of internalization, or decreased receptor affinity in acute inflammation.

The major contractile effect of ACH was through muscarinic receptors on the muscle, as shown by a dramatic drop in the response of both saline and acute tissue in the presence of atropine (Figure 9 a and 9 b). Saline and acute tissue responses to ACH in the presence of HEX, conditions which allow only muscarinic-mediated responses, were similar (Figure 9 a and 9 b). Therefore, unlike the TK receptors, muscarinic receptors on the muscle appeared unchanged in acute inflammation.

Chronic Changes

The physical appearance of the chronically inflamed colon was grossly different from saline and acute treatment animals. The colon was visibly dilated and frequently accompanied by intraperitoneal adhesions. The muscle tissue was rigid and fibrotic compared to saline and acute tissue. Histology measurements in Table IV and physical characteristics, outlined in Table V, show that the chronic treatment resulted in thickening of the muscularis externa, and increased wet weight, which did not occur during acute inflammation. Additionally, passive as well as active tensions at L_o were reduced.

In chronic colitis, the muscle lost the ability to generate a maximum contraction to all stimuli. However, the sensitivity to K depolarization was no different from saline, suggesting a non-specific reduction in the contractile mechanisms, rather than altered excitability or changes in resting membrane potential. Several investigators have proposed possible mechanisms for the decreased contractility shown previously in models of chronic colitis [25, 58]. Xie et. al. observed that chronic inflammation resulted in diminished crossbridge formation between actin and myosin, as well as an absolute reduction in the levels of these proteins[62, 63]. The lower passive and active tension in chronic muscle support a similar mechanism in our study.

Another investigator postulated that decreased contraction was caused by increased resistance forces[45]. Growth factors present during chronic inflammation may increase deposition of extracellular fibrous matrix in the muscle, increasing resistive forces that must be overcome during contraction (Figure 15). Typical tracings from muscle challenged with 60 mM KCl illustrated a dramatic decrease in the rate of rise to the maximal contraction in chronic inflammation (Figure 5). The rigid and fibrotic tissue from the chronic group took a longer time to reach peak contraction. Thus, the physical characteristics and K response suggest both decreased contractile elements, as well as increased resistance forces contributed to depressed contractile response in our chronic group.

Smooth muscle from chronically inflammed animals exhibited decreased sensitivity to the receptor mediated agonists and decreased T_{max} response to all agents. The overall decreased responsiveness of the chronic muscle made assessment of changes in receptor mediated events difficult to interpret. However, maximal responses as well as sensitivities (increased EC_{50}s) to NKA, SP and ACH challenges were reduced, indicating that these reductions were mediated through changes in receptors. Furthermore, there was no evidence of cholinergic regulation of the SP and NKA response, implying a functional loss of muscarinic receptor-mediated events. Therefore, in chronic tissue, both TK and muscarinic receptors were impaired.

NEURAL CHANGES

Changes in neural input to smooth muscle may contribute to altered contractility and subsequent motility patterns during inflammation. Figure 16 summarizes some of the components within the neural compartment that may impact on the contractile state of the smooth muscle. The ENS has sensory, motor, and interneurons that can undergo changes in transmitter release, receptor density and/or function, or transmitter degradation. Additionally, there can be changes in second messenger systems or resting membrane potentials of neurons induced by either inflammatory mediators or altered neurotransmitter profiles. Changes in the ENS were assessed using a pharmacological approach. Since neurotransmitters were added exogenously, they may interact at sites within the ENS as well as directly on the muscle.

Acute Changes

Acute inflammation caused an elevation of the response to ACH, which acts at both muscarinic and nicotinic receptors within the ENS. Nicotinic receptors are located exclusively within the enteric ganglia. In saline treated animals blocking nicotinic receptors with HEX allowed a dramatic increase in the response to ACH (Figure 9 a).

Therefore, in saline treated animals, activation of nicotinic receptors mediated an inhibition of the ACH response. In acutely treated animals, blocking nicotinic receptors with HEX made no difference in the response to ACH (Figure 9 b). This suggests the level of nicotine mediated inhibition in the acute tissue was significantly reduced. Thus, the reduction in ACH response observed in the acute animals was due to a loss of nicotinic mediated inhibition.

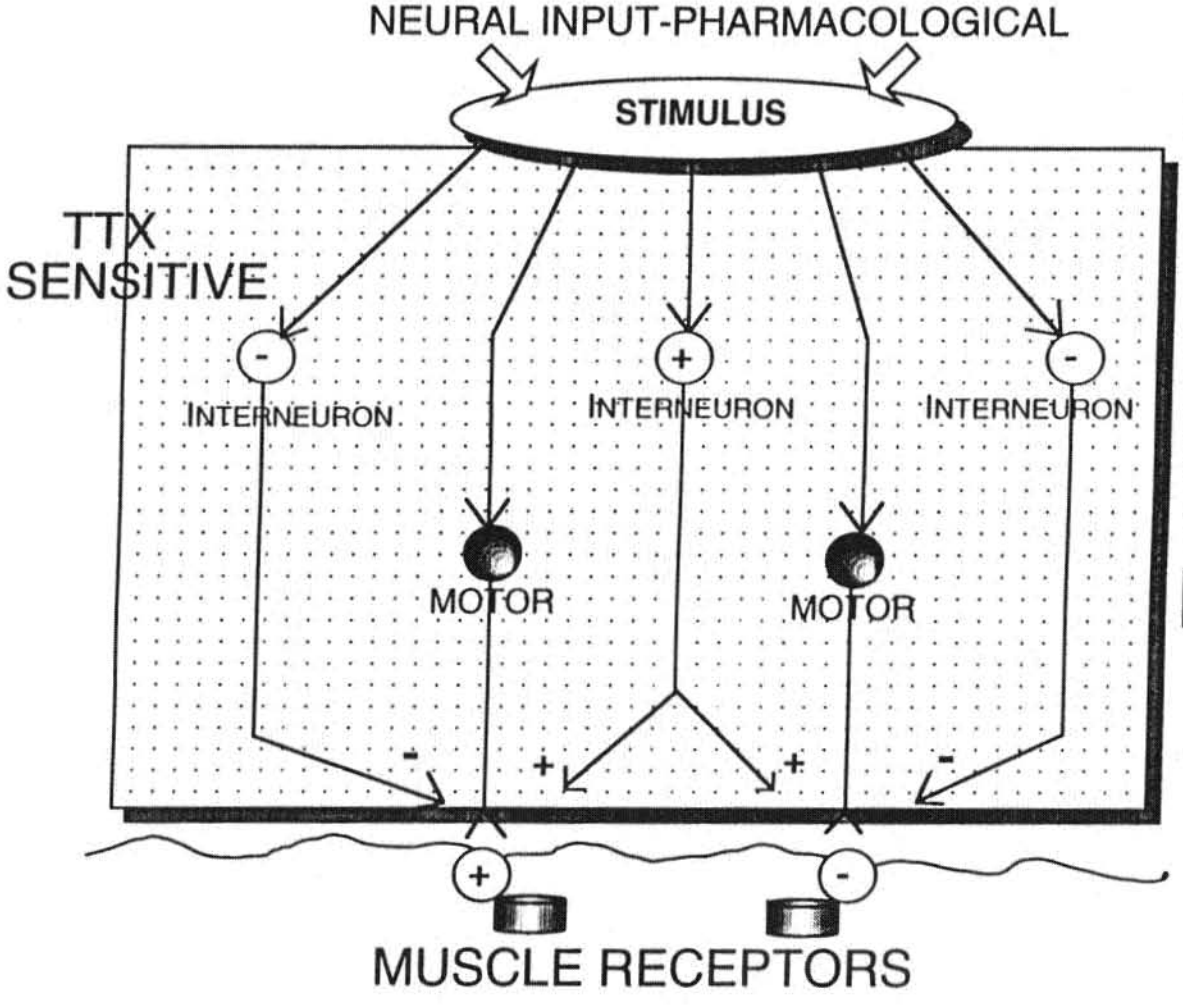
ACH stimulation of nicotinic receptors appear to release inhibitory neurotransmitters such as NO and VIP, both of which contribute to the tonic relaxed state of the muscle (Figure 1). A decrease in this neural pathway would have decreased the tonic inhibitory tone of the muscle, making the muscle more excitable. This may be an important factor contributing to the increased K sensitivity of the acutely inflamed muscle.

ACH may also bind to muscarinic receptors within the ENS. Five distinct types of muscarinic receptors have been genetically cloned (M_{1-5}), but three main subclasses have been characterized functionally; M_1, M_2 and M_3[47]. Atropine effectively blocks ACH activity at all M receptor subtypes. M_1 and M_2 receptors are located within the neural compartment, while M_3 is exclusively on the muscle[47]. M_1 is located on the nerve post synaptically, while M_2 is located presynaptically, and is thought to inhibit further release of ACH from the nerve terminal[47]. The greatest response to muscarinic activation was through the M_3 receptor at the muscle, therefore changes in neural M_1 and M_2 receptors could not be assessed from ACH response data. Thus, while we cannot document neural muscarinic changes with the ACH data, we can infer from acute atropine-dependent TK changes that muscarinic receptors within the neural compartment were altered.

During acute inflammation there were apparent atropine sensitive neural changes in the regulation of SP and NKA that were not reflected by their respective receptor-selective agonists. The fact that NK_3 receptors showed no atropine sensitive neural component discounts the theory that SP or NKA may have acted through this alternate neuronal receptor. It should be noted that all three receptor-selective agonists gave responses that were consistant with

Figure 16. Illustration showing neural components that contribute to muscle contractility.

MECHANISMS OF ENS NEURAL COMPONENT



POSSIBLE NEURAL ALTERATIONS

1. Receptor changes
   - -affinity
   - -density
   - -function
2. Second messenger systems
3. ↑ or ↓ neurotransmitter release
   - -LTD_4 ↑
4. ↑ or ↓ neurotransmitter degradation
5. ↑ or ↓ RMP of nerves
   - -inhibitory or excitatory post synaptic potentials

an exclusive action at the muscle. None showed a strong neural component. As mentioned before, the literature states that NK_1 and NK_3 receptors exist within the neural compartment, while NK_2 is primarily at the muscle. These studies were done using different selective agonists, different regions of the gut, and different animal species, so it is conceivable from our studies that the distal colon of the rat expresses all TK receptor subtypes primarily at the muscle. However, given the complexity of the ENS and the important role of the TKs in the neural circuitry, this is unlikely.

We can conclude one of two things from our data. First, if we believe NK_1, NK_2 or NK_3 receptors are not expressed in nerves, as suggested from our selective agonist data, then we have to conclude that SP and NKA acted through another yet undefined neuronal NK receptor subtype. This is improbable, but not impossible. An alternate theory is that the selective agonists we chose were active primarily at the muscle receptors and not as potent at the neural receptors.

There is variability in the NK_1 and NK_2 receptors in their pharmacologic response to commercially developed agonists and antagonists. Agonists appear to be comparably active at NK_1 receptors, but antagonists have shown species differences in their binding affininty for the NK_1 receptor[2]. NK_2 receptor subtype pharmacology also exhibits species dependency. The pharmacologically defined NK_{2A} receptor is more prevalent in human colon, while the NK_{2B} receptor subtypes are preferentially expressed in rat intestine[5, 18]. β-alanine, our selective NK_2 agonist, acts as a full agonist at either receptor subtype[35, 50]. Therefore, while we can say that our pharmacological agonists were appropriate for the rat colitis model, we cannot rule out the potential for another yet undefined pharmacological subtype of TK receptor on the nerves.

Studies using three TK receptor antagonists to block SP-induced release of ACH showed minimal effect of SP block on the neuronal receptors [13]. Furthermore, NK_1 antagonists were not effective at blocking SP-induced neuronal depolarizations from either rat or guinea pig myenteric neurons[15, 61]. These studies indicate the NK_1 receptors on the nerves are different from their target cell

counterpart[14]. Our data would suggest that similar to NK_1 antagonists, the selective receptor agonists chosen have minimal activity at both NK_1 and NK_2, and possibly NK_3 neuronal receptors in our rat model of colitis. Therefore, we cannot rule out the possibility that SP and NKA are working through their respective neural receptors to cause the atropine sensitive changes evident during acute inflammation.

Chronic Changes

Cholinergic mediated neural changes in the chronic group are more difficult to discern than those related to changes at the muscle, due to the low non-specific contractile responses. Neurally-mediated alterations may occur, but their detection is impaired by changes at the muscle. Evidence of nicotinic inhibition of ACH response is totally absent in chronic tissue. In fact, response curves in the presence of HEX suggest a nicotine mediated excitation ($P=0.054$, Figure 9 c). In the saline group, the nicotinic mediated response to ACH may be primarily inhibitory, with a small excitatory component. During the inflammatory process, the nicotinic receptor- mediated inhibition declines until, only the excitatory component dominates in chronic inflammation. Alternatively, the excitatory nicotinic component may be upregulated in an attempt to maintain normal smooth muscle contractility. Additionally, chronic inflammation results in a total loss of the atropine sensitive neural component influencing the TKs (Figure 7 a and 7 b). Therefore, during the progression of the inflammation into the chronic stage, nicotinic receptor- mediated inhibition and muscarinic receptor-mediated activity were lost, while an excitatory nicotinic component was revealed.

MECHANISMS FOR THE OPPOSING TK CHANGES DURING ACUTE INFLAMMATION

The two TKs, SP and NKA, share many similarities. They have similar amino acid sequences, act through the same second messenger system, exhibit cross reactivity at each other's receptors,

and are thought to coexist within the same synaptic vesicles[8, 14, 23]. It is a paradox then, that they show dramatically different changes in their action during acute inflammation. NKA response was higher than that of SP in saline treated tissue. SP action increased, while that of NKA decreased during acute inflammation, leaving the muscle more responsive to SP than NKA. The atropine sensitive neural influence differed between the two TKs, both in saline treated and acutely inflamed animals, and both changed during inflammation. NKA was positively influenced by muscarinic mechanisms, while SP response was attenuated in saline animals. During acute inflammation, NKA acquired a muscarinic inhibition, while SP lost muscarinic inhibition. It was the atropine sensitive neural changes of the TK response that effected the opposing changes of the TK response during acute inflammation.

NKA response changes during acute inflammation may reflect an imbalance between positive and negative atropine sensitive neural components. Figure 17 illustrates possible mechanisms that may have influenced the atropine sensitive NKA response during acute inflammation. The atropine sensitive contractile response to NKA is the net result of neural signals linked through either excitatory or inhibitory neurotransmitters (Figure 17). In normal tissue, the addition of atropine decreases the NKA response, indicating that cholinergic release of excitatory neurotransmitters was the predominating input (refer to results; Figure 6 c). In acutely inflamed tissue, neurotransmitter release, degradation, or sensitivities are changed to make inhibitory input the predominant feature of the atropine sensitive NKA response. This effectively decreases the contractile response to NKA in acute inflammation (refer to results Figure 4 c). The addition of atropine to the acutely inflamed tissue blocks this predominant inhibition and allows an increased response to NKA (refer results Figure 6 d). Thus, while the atropine sensitive neuronal component is excitatory in saline treated rats, it is inhibitory in the acute treatment group. It appears that the changes in the atropine sensitive NKA response is caused by the imbalance between the inhibitory and excitatory signals. Any inflammatory mediated alterations that increase the inhibitory

Figure 17. Illustration showing hypothetical mechanisms of NKA atropine sensitive contractile response.

HEALTHY
INFLAMED
NKA
NKA
NK2
NK2
NK2
NK2
ACH
ATROPINE
M1
ACH
ATROPINE
M1
NO/VIP
NO/VIP
NK2
NK2
ACH
ATROPINE
M3
ACH
ATROPINE
M3

component, or decrease the excitatory component could be plausible explanations for the imbalance.

SP atropine sensitive neural component differed from that of NKA. Normal tissue exhibited an atropine sensitive inhibition of SP response (refer to results Figure 6 a) Acute inflammation caused a loss of this inhibition, resulting in an increased response to SP (refer to results Figure 4 a and 6 b). The diagram in Figure 18 illustrates the changes in SP atropine sensitive neural regulation and suggests possible mediating transmitters. NO and VIP are likely candidates as inhibitory transmitters of the SP atropine sensitive response. Therefore, unlike the imbalanced signal of the atropine sensitive NKA response, the proposed atropine sensitive SP mechanism is based on a loss of inhibitory input.

Changes in both SP and NKA response during inflammation were mediated in part by changes in atropine sensitive neural muscarinic receptors. The muscarinic changes that occurred may be the same for NKA and SP, but the modulation of the TK responses differed. The ultimate contractile event is entirely dependent on the circuitry involved between the TK receptor and the motor response (the muscle contraction). A change in the ACH release or a change in sensitivity of M_1 receptors could effect either a positive or negative influence on the TKs depending on the route through either excitatory or inhibitory interneurons (Figure 16). It is well known that the TKs can initiate the release of ACH[28, 48]. Released ACH may have acted directly on M_3 muscle receptors which is the proposed scenario for excitatory input of the NKA response. Alternatively, it may have stimulated post synaptic M_1 receptors causing release of NO/VIP, as in the proposed inhibitory input of NKA or SP responses. There is documentation in the literature of ACH mediated NO release within the ENS[60]. Therefore, the changes at the muscarinic receptors could be similar for NKA and SP responses, but the paths through the ENS must differ, because the ultimate regulation of the atropine sensitive neural components move in opposing directions during acute inflammation.

Figure 18. Illustration showing hypothetical mechanisms of SP atropine sensitive contractile response.

HEALTHY
SP
NK1
ACH
ATROPINE
M1
NO/VIP
NK1
INFLAMED
SP
NK1
ACH
ATROPINE
M1
NO/VIP
NK1

SUMMARY AND CONCLUSION

Our studies document both progressive and tissue-specific changes that occur during the inflammatory process in the rat colon. In the initial stages of inflammation, despite decreased NK_1 activity at the muscle, the response to SP is increased primarily due to the loss of an inhibitory neural influence. Even in the presence of a more excitable muscle, the response to NKA is decreased in acute inflammation due to a combined decrease in the NK_2 receptor activity at the muscle, and the acquisition of a predominantly inhibitory neural component. All TK receptor activity is decreased at the level of the muscle. None of the TK receptors appeared to be strongly expressed on neurons, either in normal or acutely inflamed tissue. This may reflect the inability of our selective agonists to activate neural receptors. Response to ACH is increased in acute inflammation, not through a detectable change in muscarinic receptors, but through a loss of nicotinic inhibitory neural input.

Chronic changes caused reductions in all receptor-mediated activity. The ability of the muscle to mount a full contractile response was impaired in the chronic tissue. This was probably not due to less excitability, but rather to decreases in contractile proteins combined with increased resistance from surrounding tissue. The sequential progression of the circular smooth muscle from the more contractile state into a less responsive tissue correlates well with the increased transit followed by decreased transit during *in vivo* inflammation studies. Therefore, the changes in muscle contractility documented in our study may contribute to the discomfort and diarrhea of those patients with IBD.

BIBLIOGRAPHY

1. Allescher, H.D. and S. Ahmad, *Neuropeptide Function in the Gastrointestinal Tract.* Postulated Physiological and Pathophysiological Roles on Motility, ed. E.E. Daniel. 1991, Boca Raton, FL: CRC Press, Inc. 333.

2. Appell, K.C., B.J. Fragale, J. Loscig, S. Singh, and B.E. Tomczuk, *Antagonists that demonstrate species differences in neurokinin-1 receptors.* Mol. Pharmacol. 41: 772-778,1992.

3. Blennerhassett, M.G., P. Vignjevic, D.L. Vermillion, and S.M. Collins, *Inflammation causes hyperplasia and hypertrophy in smooth muscle of rat small intestine.* Am. J. Physiol. 262(Gastrointest. Liver. Physiol. 25): G1041-G1046,1992.

4. Brenner, K.G. and C.S. Melnyk, *Ulcerative colitis.* Inflammatory Bowel Diagnosis and Treatment., ed. G. Gitnick. 1991, New York, NY: Igaku-Skoin. 89-100.

5. Brown, M.C.S., M. Biagi, A.R. Renzette, P. Rovero, M. Criscuoli, and J. Mizrahi, *[^{125}I]His-neurokinin A binds selectively to NK_2 receptors of the B-type in rat small intestine smooth muscle membranes.* Eur. J. Pharmacol.(Mol. Pharmacol. Section) 227: 163-171,1992.

6. Chuang, D., J.L. Conklin, and G. Hammer, *Enteric neural modulation of slow-wave activity in cat colon.* Dig. Dis. Sci. 36(6): 719-726,1991.

7. Collins, S.M., P.A. Blennerhassett, M.G. Blennerhassett, and D.L. Vermillion, *Impaired acetylcholine release from the myenteric plexus of Trichinella-infected rats.* Am. J. Physiol. 257(Gastrointest. Liver Physiol. 20): G898-G903,1989.

8. Deacon, C.F., A.R. Nau, and J.M. Conlon, *Conversion of neuropeptide K to neurokinin A and vesicular colocalization of neurokinin A and substance P in neurons of the guinea pig small intestine.* J. Neurochem. 48: 141-146,1987.

9. Djokic, T.D., K. Sekizawa, D.B. Borson, and J.A. Nadel, *Neutral endopeptidase inhibitors potentiate substance P induced contraction in gut smooth muscle.* Am. J. Physiol. 256(Gastrointest. Liver. Physiol. 19): G39-G43,1989.

10. Domoto, T., J. Jury, I. Berezin, J.E.T. Fox, and E.E. Daniel, *Does Substance P comediate with acetylcholine in nerves of opossum esophageal muscularis mucosa?* Am. J. Physiol. 245(Gastrointest. Liver Physiol. 8): G19-G28,1983.

11. Evangelista, S. and A. Meli, *Influence of capsaicin sensitive fibers on experimentaly induced colitis in rats.* J. Pharm. Pharmacol. 41: 574-575,1989.

12. Eysselein, V.E., M. Reinshagen, F. Cominelli, C. Sternini, W. Davis, A. Patel, C.C. Nast, D. Bernstein, K. Anderson, H. Khan, and J.W.J. Snape, *Calcitonin gene-related peptide and substance P decrease in the rabbit colon during colitis.* Gastroenterology. 101: 1211-1219,1991.

13. Featherstone, R.L., P. Fosbraey, and I.K.M. Morton, *A comparison of the effects of three substance P antagonists on tachykinin-stimulated [^{3}H]-acetylcholine release in the guinea-pig ileum.* Br. J. Pharmacol. 87: 73-78,1986.

14. Galligan, J.J., *Peptides and Enteric Neural Activity,* in Gastrointestinal Regulatory Peptides. ed. D.R. Brown. 1993, New York, NY: Springer-Verlag. 258.

15. Galligan, J.J., T. Tokimasa, and R.A. North, *Effects of three mammalian tachykinins on single enteric neurons.* Neurosci. Lett. 82: 167-171,1987.

16. Gilbert, R.J., C. Pothoulakis, and J.T. Lamont, *Effect of purified Clostridium difficile toxins on intestinal smooth muscle. I. Toxin B.* Am. J. Physiol. (Gastrointest. Liver Physiol. 19): G767-G772,1989.

17. Gilbert, R.J., G. Triadafilopoulous, C. Pothoulakis, C. Giampaolo, and J.T. Lamont, *Effect of purified Clostridium difficile toxins on intestinal smooth muscle. I. Toxin A.* Am. J. Physiol. (Gastrointest. Liver Physiol. 19): G759-G766,1989.

18. Giuliani, S., G. Barbanti, D. Turini, L. Quartara, P. Rovero, A. Giachette, and C.A. Maggi, *NK_2 tachykinin receptors and contraction of circular muscle of the human colon: characterization of the NK_2 receptor subtype.* Eur. J. Pharmacol. 203: 365-370,1991.

19. Goldhill, J., V. Pinerio-Carrero, R. Sjogren, and T. Shea-Donohue, *Tachykinin-mediated intestinal contraction is increased in experimental ileitis.* Gastroenterology (In Press). 1994.

20. Goldhill, J.M., F.D. Finkelman, S.C. Morris, and T. Shea-Donohue, *Neural control of mouse small intestinal longitudinal muscle: interactions with inflammatory mediators.* J. Pharmacol. Exp. Ther. 274(1): 72-77,1994.

21. Gordon, A.R. and M.J. Siegman, *Mechanical properties of smooth muscle. I. Lenth-tension and force-velocity relations.* Am. J. Physiol. 221(5): 1243-1249,1971.

22. Goso, C., S. Evangelista, M. Tramontana, S. Manzini, P.M. Blumberg, and A. Szallasi, *Topical capsaicin administration protects against trinitrobenzene sulfonic acid induced colitis in the rat.* Eur. J. Pharmacol. 249: 185-190,1993.

23. Grider, J.R., *Peptidergic Regulation of Smooth Muscle Contractility.* Gastrointestinal Regulatory Peptides. ed. D.R. Brown. 1993, New York, NY: Springer-Verlag. 285.

24. Grider, J.R. and G.M. Makhlouf, *Contraction mediated by Ca^{++} release in circular and Ca^{++} influx in longitudinal intestinal muscle cells.* J. Pharm. Exp. Ther. 244(2): 432-437,1988.

25. Grossi, L., K. McHugh, and S.M. Collins, *On the specificity of altered muscle function in experimental colitis in rats.* Gastroenterology. 104: 1049-1056,1993.

26. Helstrom, P.M., K.S. Murthy, J.R. Grider, and M. Makhlouf, *Coexistence of three tachykinin receptors coupled to Ca^{++} signaling pathways in intestinal muscle cells.* J. Pharmacol. Exp. Ther. 270(1): 236-243,1994.

27. Holmgren, S., *The Comparative Physiology of Regulatory Peptides.* Gut Motility, ed. S. Holmgren. 1989, New York, NY: Chapman and Hall. 245.

28. Holzer, P., P.C. Emson, L.L. Iversen, and D.R. Sharman, *Regional differences in the response to substance P of the longitudinal muscle and the concentration of substance P in the digestive tract of the guinea-pig.* Neuroscience. 6(7): 1433-1441,1981.

29. Holzer, P., E.H. Livingston, and P.H. Guth, *Sensory neurons signal for an increase in rat gastric mucosal blood flow in the face of pending acid injury.* Gastroenterology. 101: 416-423,1991.

30. Huizinga, J.D., G. Chang, N.E. Diamant, and T.Y. El-Sharkawy, *Electrophysiological basis of excitation of canine colonic circular muscle by cholinergic agents and substance P.* J. Pharm. Exp. Ther. 231(3): 692-699,1984.

31. Janowitz, H.D. and K. Mauer, *Crohn's Disease*. Inflammatory Bowel Diagnosis and Treatment, ed. G. Gitnick. 1991, New York, NY: Igaku-Khoin. 101-112.

32. Kim, Y.C. and P.M. Hellstrom, *Identification of mechanisms for duodenal contraction induced by tachykinins in the rat.* J. Gastrointest. Mot. 5: 97-106,1993.

33. Koch, T.R., M.A. Carney, and V.L.W.Go, *Distribution and quantitation of gut neuropeptides in normal intestine and inflammatory bowel diseases.* Dig. Dis. Sci. 32(4): 369-376,1987.

34. Laburfthe, M., P. Kitabgi, A. Couvineau, and B. Amiranoff, *Gastrointestinal Regulatory Peptides*. Peptide Receptors and Signal Transduction in the Digestive Tract, ed. D.R. Brown. 1993, New York, NY: Springer-Verlag. 148.

35. Maggi, C.A., S. Guiliani, L. Ballati, P. Robero, L. Abelli, S. Manzini, A. Giachette, and A. Meli, *In vivo pharmacology of [β-ala^8]-NKA(4-10), a selective NK-2 tachykinin receptor agonist.* Eur. J. Pharmacol. 177: 81-87,1990.

36. Maggi, C.M., R. Patacchini, A. Giachetti, and A. Meli, *Tachykinin receptors in the circular muscle of the guinea-pig ileum.* Br. J. Pharmacol. 101: 996-1000,1990.

37. Mantyh, C.R., T.S. Gates, R.P. Simmerman, M.L. Welton, E.P. Passara, S.R. Vigna, J.E. Maggio, L. Kruger, and P.W. Mantyh, *Receptor binding sites for substance P, but not for substance K or neuromedin K, are expressed in high concentrations by arterioles, venules and lymph nodules in surgical specimens obtained from patients with ulcerative colitis and Crohn's disease.* Proc. Nat. Acad. Sci. USA. 85: 3235-3239,1988.

38. Masella, J.S., K.A. Davis, and M.G. Blennerhassett, *Decreased cholinesterase activity with increased acetylcholine synthesis in*

inflamed rat intestine: Potential for increased cholinergic influence. Gastroenterology. 108(4): A872,1995.

39. Mazumdar, S. and K.M. Das, *Immunocytochemical localization of vasoactive intestinal peptide and substance P in the colon from normal subjects and patients with inflammatory bowel disease.* Am. J. Gastroeenterol. 1987(2): 176-181,1992.

40. Mendeloff, A.I., *Inflammatory bowel disease epidemiology in the United States.* Inflammatory Bowel Disease. ed. P.B. Goebell and H Malchow. 1987, Lancaster, England: MTP Press Limited. 243-244.

41. Morita, K., R.A. North, and T. Tokimasa, *Muscarinic presynaptic inhibition of synaptic transmission in myenteric plexus of guinea-pig ileum.* J. Physiol. 333: 141-149,1982.

42. Morris, G.P., P.L. Beck, M.S. Herridge, W.T. Depew, M.R. Szewczuk, and J.L. Wallace, *Hapten-induced model of chronic inflammation and ulceration in the rat colon.* Gastroenterology. 96: 795-803,1989.

43. Payan, D.G., *Neuropeptides and inflammation: the role of substance P.* Ann. Rev. Med. 1989. 40: 341-352,1989.

44. Percy, W.H., M.B. Burton, Y. Jacobowitz, and R. Burakoff, *An investigation in vitro of the properties of the individual muscle layers of the rabbit colon in an induced colitis.* Effects of Immune Cells and Inflammation on Smooth Muscle and Enteric Nerves. eds. W.J. Snape and S.M. Collins. 1991, Boca Raton, FL: C.R.C. Press. 95-108.

45. Percy, W.H., M.B. Burton, K. Rose, V. Donovan, and R. Burakoff, *In vitro changes in the properties of rabbit colonic muscularis mucosae in colitis.* Gastroenterology. 104: 369-376,1993.

46. Pons, L., M.T. Droy-LeFaix, and L. Bueno, *Leukotriene D_4 participates in colonic transit disturbances induced by intracolonic*

administration of TNBS in rats. Gastroenterology. 102(1): 149-156,1992.

47. Rang, H.P., M.M. Dale, J.M. Ritter, and P. Gardner, *Chapter 6: Cholinergic Transmission.* Pharmacology, Third Edition. ed. H.P. Rang, M.M. Dale, J.M. Ritter, and P. Gardner. 1995, New York, NY: Churchill Livingston Inc. 117-147.

48. Regoli, D., P. D'orleans-Juste, E. Escher, and J. Mizrahi, *Receptors for substance P. I. The pharmacological preparations.* Eur. J. Pharmacol. 97: 161-170,1984.

49. Reinshagen, M., A. Patel, M. Sttili, C. Nast, W. Davis, K. Mueller, and V.E. Eysselein, *Protective function of extrinsic sensory neurons in acute rabbit experimental colitis.* Gastroenterology. 106: 1208-1214,1994.

50. Rovero, P., N.E. Rhaleb, S. Dion, N. Rouissi, C. Tousignant, S. Telemaque, G. Drapeau, and D. Regoli, *Structure activity studies of neurokinin A.* Neuropeptides. 13: 263-270,1989.

51. Sanders, K.M. and T.K. Smith, *Motor neurones of the submucous plexus regulate electrical activity of the circular muscle of canine proximal colon.* J. Physiol. 380: 293-310,1986.

52. Sarna, S.K., *Physiology and Pathophysiology of colonic motor activity (Part one of two).* Dig. Dis. Sci. 36(6): 827-862,1991.

53. Sarna, S.K., *Physiology and Pathophysiology of colonic motor activity (Part two of two).* Dig. Dis. Sci. 36(7): 998-1018,1991.

54. Sartor, R.B., W.J. Cromartie, D.W. Powel, and J.H. Schwab, *Granulomatous enterocolitis induced in rats by purified bacterial cell wall fragments.* Gastroenterology. 89: 587-595,1985.

55. Schmidt, P., T.N. Rasmussen, and J.J. Holst, *Nervous control of the release of substance P and neurokinin A from the isolated perfused porcine ileum.* J. Auto. Nerv. Syst. 38: 85-96,1992.

56. Shorter, R.G., K.A. Huizenga, and R.J. Spencer, *A working hypothesis for the etiology and pathogenesis of nonspecific inflammatory bowel disease.* Digestive Diseases. 17(11): 1024-1032,1972.

57. Sjogren, R.W., C. Colleton, and T. Shea-Donohue, *Intestinal myoelectric response in two different models of acute enteric inflammation.* Am. J. Physiol. 254(Gastrointest. Liver. Physiol. 30): G1-G9,1994.

58. Snape, W.J. and H.W. Kao, *Role of inflammatory mediators in colonic smooth muscle function in ulcerative colitis.* Dig. Dis. Sci. 33(3): 65S-70S,1988.

59. Ward, M., *The pathogenesis of Crohn's disease.* Lancet. 2: 903-905,1977.

60. Wiklund, C.U., N.P. Wiklund, and L.E. Gustafsson, *Modulation of neuroeffector transmission by endogenous nitric oxide: a role for acetylcholine receptor-activated nitric oxide formation, as indicated by measurements of nitric oxide/nitrite release.* Eur. J. of Pharmacol. 240: 235-242,1993.

61. Willard, A.L., *Substance P mediates synaptic transmission between rat myenteric neurons in cell culture.* J. Physiol. 426: 453-471,1990.

62. Xie, Y.N., W.T. Gerhoffer, S.N. Reddy, F. Cominelli, V.E. Eysselein, and W.J. Snape, *An abnormal rate of actin myosin cross-bridge cycling in colonic smooth muscle associated with experimental colitis.* Am. J. Physiol. 262(Gastrointest. Liver. Physiol. 25): G921-G92,1992.

63. Xie, Y.N., W.T. Gerthoffer, H. Ennes, and W.J. Snape, *Effect of mucosal inflammation on the major contractile proteins in colonic smooth muscle.* Motility and Nerve-Gut Interactions: A535,1992.

64. Yamada, T., K. Fijimoto, P. Tso, T. Fijimoto, T.S. Gaginella, and M.B. Grisham, *Misoprostol accelerates colonic mucosal repair in acetic acid induced colitis.* J. Pharmacol. Exp. Ther. 260: 313-318,1992.

65. Yamada, T., S. Marshall, R.D. Specian, and M.B. Grisham, *A comparative analysis of two models of colitis in rats.* Gastroenterology. 102: 1524-1534,1992.